

AN INTRODUCTION TO INDUSTRIAL MYCOLOGY

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FOREWORD

It is a truism to say that were all types of micro-organisms suddenly to die out, human and animal life as we know it to-day would be impossible. Dead vegetable and animal remains are continuously being broken down by biochemical reactions brought about by micro-organisms, until their constituent elements are returned to the economy of nature as carbon dioxide, ammonia, nitrates, etc., to recommence the synthetic cycle for which the green plants are responsible. The part played by the lower fungi, or "moulds" as they are more commonly known, in this chain of degradative processes is one of paramount importance.

Further, in all industries handling organic materials, e.g. those concerned with food production, leather, textiles, wood, pharmaceuticals, etc., the danger of spoilage through growth of moulds is one of which all those engaged in these industries are acutely aware. Thus the control and prevention of mould growth becomes a major problem to which, in the interests of increased efficiency, more and more attention is being given.

The harnessing of moulds for beneficial purposes, long established in some industries, as for example in the manufacture of Stilton, Gorgonzola and other types of cheese, has in recent years followed some very interesting lines. Thus citric acid, until this century obtained exclusively from the juice of citrus fruits, is now being made in thousands of tons per annum and in many different countries by growing chosen strains of the common black mould *Aspergillus niger* on sugar solutions, under carefully controlled conditions. The study of the biochemical changes, almost bewildering in their diversity, which can be brought about by moulds, is now a rapidly developing branch of biochemistry, and is attracting the attention of scientific workers in different parts of the world.

To those who are actively concerned in any of the industrial or scientific pursuits I have mentioned—and many more examples could be given if these were needed—it is scarcely necessary for me to point out the paramount importance of some knowledge of the moulds themselves, and the more detailed and accurate this knowledge is, the better. Thousands of different species of moulds have been described and their differences in response to a particular environment, their tolerance of adverse conditions, and their biochemical characteristics, are almost as varied as their numbers are great. There are in existence many admirable text-books on mycology which will probably meet the needs of the student who has had an adequate training in botany. But to those with little or no botanical knowledge, and particularly for those who are faced for the first time with an industrial problem of “mould” control, the lack of an adequate text-book setting forth in simple language the facts of the subject, is a very real lack. To these and to any others who wish to acquire a first-hand knowledge of the common “moulds” I most warmly recommend this book written by my colleague, Mr. George Smith. The subject-matter of the book forms the basis of a course of lectures and practical work given by the author as part of the course to students working in this School for the post-graduate Academic Diploma in Bacteriology of the University of London. Readers of Mr. Smith’s book will, I think, find it easy to read and stimulating to study, and will, I hope and believe, particularly appreciate, as I do, the really beautiful photomicrographs which form a very important part of the book.

H. RAISTRICK.

LONDON SCHOOL OF HYGIENE
AND TROPICAL MEDICINE.

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PREFACE

This book is intended to assist those who are commencing the study of "moulds" rather than of fungi in general. There is already an extensive literature of systematic mycology, plant pathology and medical mycology, but there has been up to the present no book in English, apart from highly specialized monographs, dealing particularly with the fungi which are of importance in industry.

Sufficient general mycology is included to enable the student to follow up the subject in the standard text-books. The major portion of the book, however, consists of descriptions and illustrations of most of the genera of moulds which are of regular occurrence in industrial products, with more detailed consideration of the genera which are of greatest importance. Chapters on laboratory methods are sufficiently detailed to enable those who have had no previous biological training, and who are unable to get personal instruction, to work from the beginning along the right lines.

Many, probably the majority, of those who are called upon to undertake the solution of problems connected with moulds in industry are chemists, most of whom have had no training in botany and who find it difficult to learn the special terminology of mycological literature. Throughout the book, therefore, I have endeavoured to explain all such terms and usages as are like to be unfamiliar to the non-botanical reader.

All the figures, except Fig. 71, are from original photomicrographs, this type of illustration being, in my opinion, more suitable than line drawings for the use of beginners. With few exceptions they are all at certain precise and selected magnifications and are readily comparable one with another. I am grateful for permission to include certain of my illus-

trations which have previously been published—Figs. 23 and 24 in *The Journal of Botany*; Figs. 67, 73, 74, 76, 81, 84, 85, 88, 89, 90, 91 and 96 in *The Journal of the Textile Institute*; Figs. 105 and 106 in *Transactions of the British Mycological Society*.

To the many colleagues and workers in other institutions who have supplied infected materials and cultures, or who have made practical suggestions, I tender my thanks. I am also greatly indebted to Messrs. Boardman & Baron, Ltd., of Great Harwood, for permission to make free use of the large number of photomicrographs taken in their laboratories.

G. S.

July 1938.

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INDUSTRIAL MYCOLOGY

CHAPTER I

INTRODUCTION

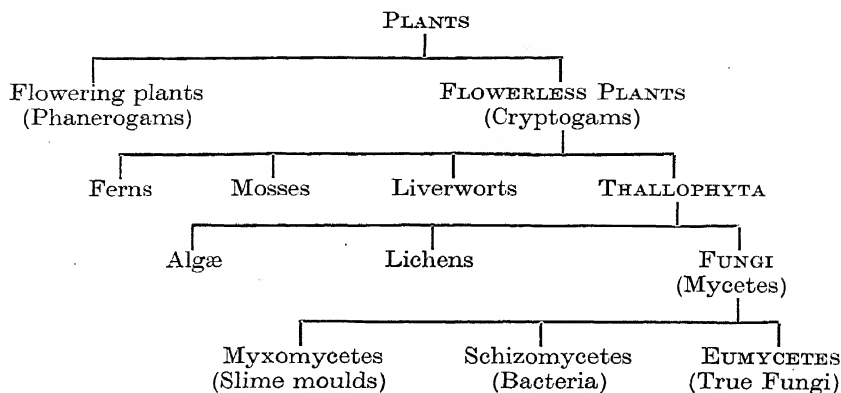
Mycology is the science of the Fungi, an enormous group of organisms of very varied form and habit. The Fungi vary in size from the large toadstools, puffballs and bracket fungi to forms which are contained within a single cell of a plant; there are species which are edible and others which are amongst the deadliest of poisons; there are very rare species and forms which are to be found almost everywhere; some species are parasites of plants and animals whilst others inhabit the soil and break down dead organic material to form plant food; there are fungi which do incalculable damage to the products of man's labour and nearly related forms whose activities are harnessed for the production of substances of great commercial value. The fungi which are the concern of Industrial Mycology are a somewhat heterogeneous collection from amongst the various orders and families, most of them of common and widespread occurrence and nearly all of them of microscopic size.

The Fungi are usually classified as part of the plant kingdom and Table I shows their relationships to other forms of plant life.

The Thallophyta are plants which show no differentiation into root, stem and leaf but whose vegetative structure is known as a thallus. This may be quite unsegmented, as in some of the simplest fungi, or may show considerable specialization of structure with corresponding specialization of function.

The Fungi are distinguished from the Algæ in showing a

TABLE I



complete lack of chlorophyll, the green colouring matter which enables plants to synthesize carbohydrates from carbon dioxide and water in the presence of sunlight. Some mycologists, on this ground, regard the Fungi as organisms quite distinct from plants and belonging neither to the plant nor animal kingdoms. Typical plants utilize simple substances and build them up into substances of greater complexity; in particular they utilize carbon dioxide, to form starch, cellulose, fats and the like, and liberate oxygen. Animals, on the whole, break down organic matter from the complex to the simple and differ fundamentally from plants in that they absorb oxygen and liberate carbon dioxide. Fungi resemble plants in structure and in lack of means of locomotion but are more related to animals in that they require oxygen and that, invariably, one of the products of metabolism is carbon dioxide. A good introduction to this question of phylogeny is given in a popular publication by Ramsbottom (1929), and the subject is further developed in most of the monographs on systematic mycology. It therefore need not be elaborated here.

The Lichens are compound organisms, consisting of algæ and fungi in symbiotic relationship. Their study is a separate branch of science, since the fungus-alga association is so close that the Lichens may be classified into genera and species just as if they were single organisms, and the fungi themselves are unknown apart from their algal associates. Those who are

interested in this group should consult the excellent monograph by Miss A. L. Smith (1918, 1926), or her text-book (1921).

The Bacteria, comprising, in the above table, one of the three classes of fungi, are the province of the bacteriologist. Whether they belong with the fungi at all is a moot point, but their consideration is outside the scope of this book.

The Myxomycetes have a somewhat varied history, having been claimed by both botanists and zoologists. The consensus of present opinion is that they are certainly not fungi and are probably classed more correctly as Mycetozoa. There are several monographs dealing with these interesting organisms, the best for British students being that of Lister (1925).

Mycology then, in its modern sense, is concerned only with the Eumycetes or true fungi.

General Morphology and Classification

Most of the common mould fungi may be propagated by transferring any part of the plant to a fresh substratum, but the normal development of a thallus commences with the germination of a *spore*. Germination is the term commonly employed, but its use in this connection must not be confused with the more accurate use of the word as applied to seeds of ordinary plants. Spores of fungi are of different shapes and sizes, they may be one- or many-celled, but in no case does a spore contain a true germ or plant embryo. A spore consists of an outer wall, the epispore, which may be smooth, pitted or roughened by small projections, and an inner wall, the endospore, enclosing a mass of protoplasm in which drops of oily or fatty material and, with special staining methods, one or more nuclei may be distinguished, but which is otherwise little differentiated. On reaching a favourable situation the spore swells and then puts out one or more *germ-tubes*. The germ-tube elongates and, in most cases, becomes richly branched, forming a network of filaments (*hyphæ*, singular *hypha*) collectively known as a *mycelium*, which may penetrate the substratum, grow on the surface or may be partly submerged and partly serial. Later, in all but a few cases, the fungus provides for propagation of the species by putting forth spore-bearing hyphæ or by building up special fruit-bodies in which the spores are formed. The methods of spore formation and the characteristics of the spores and

of the spore-bearing organs form the basis of all systems of classification of fungi.

There is no uniformity as regards classification. The terms used to denote rank, such as class, order, alliance, family are used in various senses by different authorities and the boundaries of the groups are by no means equivalent in the usages of various authors. Probably the most useful system for the beginner and for the technologist is that adopted in Rabenhorst's *Kryptogamenflora* and this system will, in the main essentials, be followed throughout the present book.

The Eumycetes are divided into 2 main groups and these into 5 classes, as follows :

- I. Hyphæ typically non-septate . . . PHYCOMYCETES.
 - A. Sexual fructification by oospores ;
asexual spores often motile . . . Class 1, OOMYCETES.
 - B. Sexual fructification by zygo-
spores ; asexual spores non-
motile, borne in sporangia or
as conidia Class 2, ZYGOMYCETES.
- II. Hyphæ typically septate . . . MYCOMYCETES.
 - A. Principal spores endogenous, in
asci ; accessory fruit forms
often present Class 3, ASCOMYCETES.
 - B. Principal spores exogenous, on
basidia Class 4, BASIDIOMYCETES.
 - C. Asci and basidia unknown ; pro-
pagation by asexual spores only . . . Class 5, FUNGI IMPERFECTI.

Several of the terms used require explanation. The first, main sub-division of the Fungi is based on the presence or absence of cross-walls (*septa*) in the hyphæ. In the Phycomycetes septa are rarely present except in the fruiting organs, though they are occasionally found in old cultures. Fig. 1 shows vegetative hyphæ of a species of *Rhizopus*, almost root-like in appearance, the whole being part of one enormously elongated and branched cell. If a young, vigorously growing culture of the very common mould, *Rhizopus nigricans*, preferably growing in a Petri dish, be examined with a low power of the microscope, it will usually be noticed that the cell contents are streaming along both the main hyphæ and the secondary branches, indicating clearly the entire absence of septa. In



FIG. 1.—Non-septate mycelium *Rhizopus nigricans*. $\times 60$.

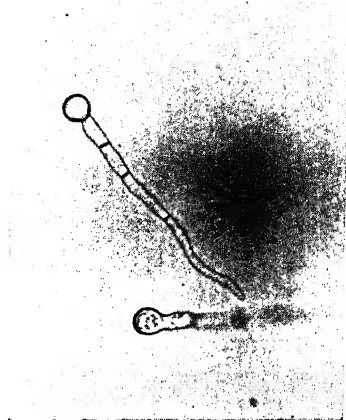


FIG. 2.—Germinating spores of *Aspergillus glaucus*, showing septation of primary hyphæ. $\times 250$.



FIG. 3.—Septate mycelium—*Alternaria tenuis*. $\times 250$.

the Mycomycetes the vegetative hyphæ usually begin to develop septa at a very early stage. Fig. 2 shows germinated spores of *Aspergillus glaucus* and the septa in the young germ-tubes are clearly visible. Fig. 3 shows the richly septate mycelium of another common mould, *Alternaria tenuis*.

The next basis of sub-division is the method of formation of the spores and here it is necessary to understand that the term "spore" is used in mycological literature with various meanings. In its wider sense it includes all the types of specialized cells, formed on or within, or cut off from, special organs, and obviously designed as a means of propagation and dissemination of the species. In its narrower sense, that used by the systematist, it is restricted to mean the product of a sexual process. The sexual union may be obvious, as in the Zygomycetes, but it is more often obscured and has only been elucidated by cytological studies, although the presence of fruit-bodies bearing a definite number of spores, usually a power of 2, is usually an indication that a sexual process is involved.

Asexual or accessory spores are of various forms and are produced on very varied types of *sporophore* (= spore-bearer). Unlike the principal or sexual spores they are produced in indefinite numbers. They may be formed within closed receptacles called *sporangia* (singular *sporangium*), or they may be cut off or bud out from special hyphæ, the *conidiophores*, the spores being then called *conidia*. Conidiophores may occur inside closed fruit-bodies (*pycnidia*), as a gelatinous or leathery layer (a *stroma*), as cushion-shaped aggregates (*sporodochia*), as compacted fascicles (*coremia*) or may be single and detached. In some fungi aerial hyphæ break up into segments which become more or less rounded and function as spores. To these the name *oidia* is given. *Chlamydo-spores*, as the name implies, are thick-walled resting spores and are formed by the swelling and thickening of single short cells in submerged vegetative hyphæ, in aerial hyphæ or even in multiseptate conidia such as those of species of *Fusarium*. *Sclerotia* are resting bodies formed of compacted masses of mycelium and are not peculiar to any particular group. They range in size from minute grains 0.1 mm. in diameter to masses weighing many pounds. The mode of occurrence and the

size, shape, septation and markings of the asexual spores are of generic significance even in the orders which are classified primarily by sexual spores, and in the great group of Fungi Imperfecti are the basis of all systems of classification.

The Oomycetes. In this class the sexual organs are readily distinguished as *antheridium* (the male organ) and *oogonium* (female organ). The oogonium, when ripe, shows part of the protoplasm aggregated into one or more *oospheres* (see Fig. 4). It is fertilized by the antheridium coming in contact with it or, in one family, the Monoblepharidaceæ, by motile male cells liberated by the antheridium. After fertilization the oospheres round up to form the *oospores*, which lie free within the oogonium (Fig. 5). Most Oomycetes also produce sporangia containing asexual spores. Many species are aquatic or grow in very damp situations and, the better to aid rapid dissemination, produce motile spores known as zoospores. The order includes a number of important plant parasites and a few parasites of animal life, the best known being species of *Saprolegnia* on fish and on insects in water, but none of the species is likely to be encountered in industrial work and, therefore, their consideration will be limited to this brief statement of their systematic position.

The Zygomycetes. In this class the sexual organs are not distinguishable as antheridium and oogonium. They may be similar in size (the usual case), or regularly dissimilar as in *Zygorhynchus*, but they are essentially alike in nature and function. Whereas in the Oomycetes the oospore is formed within the oogonium and is thus termed endogenous, the *zygospore* results from the fusion of two cells cut off from the sexual organs and is thus exogenous. Two hyphæ touch and at the point of contact develop protuberances, the *progametangia*, which gradually elongate and swell, remaining in contact themselves whilst pushing the parent hyphæ further and further apart. Septa next appear in each progametangium, the cut off portions which remain in contact being the *gametangia*. The latter fuse, the cell walls in contact disappearing, and the fusion cell becomes rounded and, usually, thick walled and dark coloured. Fig. 6 shows six stages in zygospore formation. A slight variant of this process is found in *Piptocephalis*, where the zygospore is formed in a bud put out from

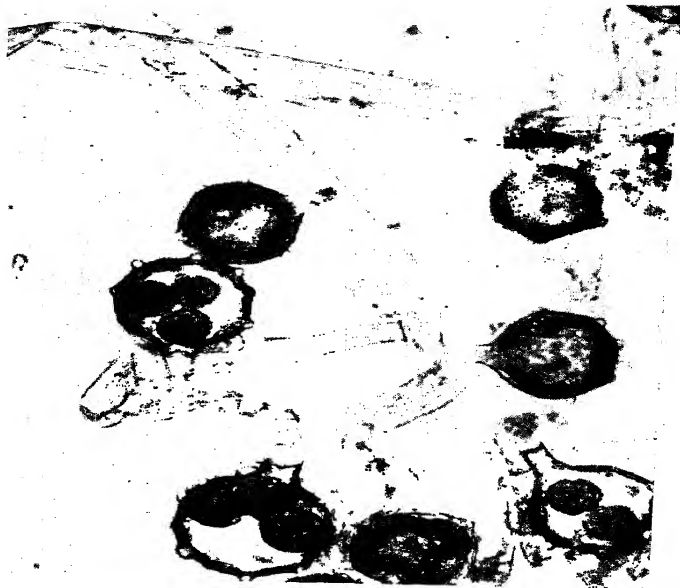


FIG. 4.—*Achlya* sp.—Oogonia with oospheres.
× 150.

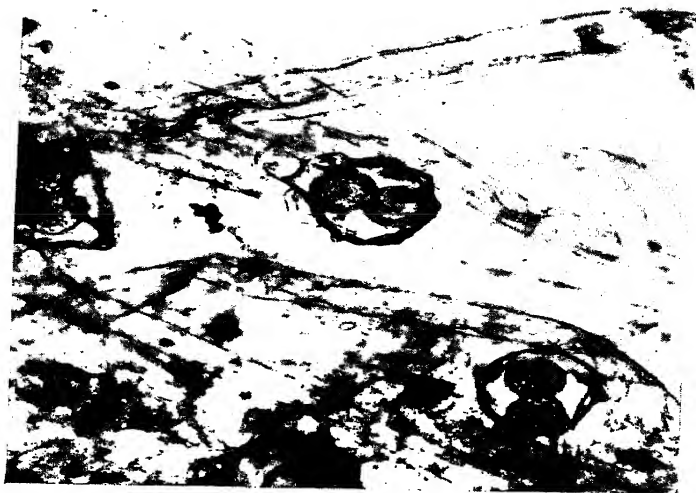


FIG. 5.—*Achlya* sp.—Oogonia with oospores.
× 150.



FIG. 6, *a-f*.—Six stages in zygospore formation—*Mucor* sp. $\times 100$.



FIG. 7.—Sporangium of *Rhizopus nigricans*. $\times 60$. The sporangial wall has burst to liberate the spores. Note the columella.

the cell which is produced by fusion of the gametangia. In some genera the zygospore is surrounded by stiff hairs or by a web of protecting hyphæ, whilst in others it lies free amongst the aerial hyphæ. In several genera which obviously belong here, zygospores have never been observed.

Asexual spores are non-motile and, in the best-known genera, are produced in globose sporangia borne on special hyphæ, the sporangiophores. Fig. 7 is a photograph of a typical sporangium of *Rhizopus nigricans*. In several genera sporangia are lacking and asexual propagation is by conidia.

The Zygomycetes include about 200 species, amongst which are a number of moulds of very common occurrence and considerable economic importance. They are considered in more detail in Chapter III.

The Ascomycetes. The typical fruit-body is a closed sac, the *ascus*, containing usually 8 spores, more rarely a smaller or larger number. The group shows a gradual transition from primitive forms which produce single, naked, globose asci to species which build up elaborate fruit-bodies with large numbers of club-shaped asci arranged in parallel series. The details of ascus formation are not readily observed and in some cases are not completely understood at the present day. Full treatment of the group from this standpoint is to be found in several modern works on systematic mycology. Asexual reproduction is by conidia, which show great diversity of form and arrangement, or by oidia. The class comprises about 15,000 species and includes both saprophytes and parasites, microscopic species and large fleshy fungi, some of which are edible. The genera which are of importance to the industrial mycologist are considered further in Chapter IV.

The Basidiomycetes. The spores which give the name to the group, the *basidiospores*, are borne exogenously on special organs, the *basidia*. In the typical species each basidium bears four spores. In the higher Basidiomycetes, the mushrooms and toadstools, the basidia are found in serrated ranks on the gills of the large fleshy sporophores and there are often specialized arrangements for ensuring the widespread distribution of the spores. The lower Basidiomycetes, including the destructive parasites known as smuts and rusts, have a more complicated life cycle, and their position in a scheme of

classification is not quite so obvious. Since most of these are obligate parasites, no attempt will be made here to discuss their relationships and life histories. They have been very extensively studied and are treated in detail in numerous monographs (see Chapter XIV). A few of the more primitive species are occasionally encountered in artificial culture, but the growths are atypical and not easily recognized.

The Fungi Imperfecti. This group comprises a very large number of fungi which have incomplete or incompletely known life cycles. Most of them are probably Ascomycetes which produce the perfect stage only under special conditions which have not yet been discovered, or which have entirely lost the power of producing asci. It is now known with certainty in a few cases that heterothallism exists amongst the Ascomycetes (see below) and it is probable that this phenomenon accounts for many other cases of apparent loss of sexuality. In the absence of perfect fructifications, on which alone a rational classification can be based, it is necessary to classify the Fungi Imperfecti on characters of the asexual spores. According to the systematists, the genera thus set up are not true genera at all, since it is known, on the one hand, that a single genus of Ascomycetes may include species with very different types of imperfect fructifications and, on the other, that the conidial forms of species belonging to widely different genera may be very similar. It follows, therefore, that a so-called "form genus," comprising a number of species with the same type of conidial fructification, may be a purely artificial grouping of fungi which are really quite unrelated. However, until real relationships are discovered, it is necessary to have a convenient classification of the enormous number of Fungi Imperfecti, as otherwise identification of any given form would be a hopeless task.

The great majority of the common fungi which are popularly known as moulds or mildew belong in this group and will be dealt with at greater length in Chapters V to VIII.

Heterothallism. It was for long a puzzle to mycologists why some species of the Mucorales regularly produced zygospores whilst others seemed to lose the power on continued culture and in still other species, which were obviously nearly related, zygospores were quite unknown. Various theories

attempting to explain the matter on a nutritional basis failed to take account of all the facts and it was not until 1904 that the riddle was solved by Blakeslee (1904, 1920). He showed that the sporangiospores from a strain of *Mucor* which was producing abundant zygosporangia gave rise, on germination, to two distinct strains of mycelium, called by Blakeslee + and - . Each strain, by itself, was incapable of giving zygosporangia but, if both strains were grown in such a way that the two mycelia could touch, zygosporangia were produced along the line of contact. It was thus obvious that mass transfers from a culture containing zygosporangia would usually result in a mixed culture of the + and - strains and zygosporangia would again be produced, but that single spore cultures would invariably lack zygosporangia and that sub-cultures made by transfer of a few spores would very often result in a pure culture of one strain only. Such species, where sexuality resides in a whole thallus, are called *heterothallic*. In contrast to these the *homothallic* moulds produce zygosporangia by contact of branches from the same mycelium and often from the same hypha. The illustrations of zygosporangium formation, Fig. 6, obviously depict a homothallic species. Both + and - strains of a number of heterothallic moulds are known and can be obtained from the various collections of type cultures, but, in a great many cases, only one strain has been found and hence zygosporangia have never been observed.

Heterothallism is most frequently encountered in species belonging in the Mucorales and it is to the numerous studies of such species that our knowledge of the phenomenon is chiefly due, but in recent years it has been shown that it is not confined to this order. Several Ascomycetes have been found to be heterothallic and in certain of the Basidiomycetes a still more complicated state of affairs exists, the four spores from a single basidium being all of different "sexes."

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CHAPTER II

TERMINOLOGY AND CLASSIFICATION

Readers who have already had some training in one of the biological sciences will have no difficulty with the system of nomenclature used by mycologists, but, to anyone without previous experience of this kind, names are apt to appear irrational and the different usages of various authorities confusing. This chapter is intended to help the beginner to understand the method which underlies the strangeness and apparent uncouthness of many mycological terms, and, at the same time, to give a broad outline of the special terminology and basis of classification.

The "unit" of any scheme of classification is the species. What exactly constitutes a species is one of the vexed questions of biology and need not be gone into but, for practical purposes, it may be taken that the term is used to denote any organism which shows recognizable and constant differences in structure from all other organisms, except, of course, from other individuals which are of the same species. A genus groups together a number of species which show a closer resemblance to each other than they do to any other species. On the same principle genera are classified into families, these into orders and orders into classes. Such a scheme of classification serves a twofold purpose. From the purely scientific point of view it represents an attempt to show how all the various species are related to each other and to other living things and it also provides a means of identification of species. Up to a point mycologists are agreed as to how the Fungi should be classified, but there are many minor points of variance between the different authorities and more fundamental differences as regards a few groups. This state of affairs is due,

partly to gaps and imperfections in our knowledge, and partly to personal differences in interpretation of observations made under difficult conditions. Also, it must be realized that there are few sharp dividing lines in Nature, almost all the groups of fungi merging imperceptibly into adjacent groups, with the result that it is often a matter of opinion as to where exactly a dividing line should be placed.

Nomenclature

Every species bears two names, that of the genus to which it belongs and its own specific name. The generic name is a Latin substantive and is always written with a capital letter, whilst the species name is adjectival, agrees with the substantive in gender and case and, normally, is not capitalized. Names should be, and frequently are, to some extent descriptive. Thus the name *Aspergillus* means "rough-head" and *Penicillium* means "broom-like," each of the names describing the gross appearance of the fruiting heads in the particular genus; *Helminthosporium* is a genus of fungi with elongate, multi-septate spores which have a somewhat fanciful resemblance to worms (Helminthes); *Rhizopus* is characterized by its production of root-like structures known as rhizoids. Specific names usually refer to minor characteristics such as colour, odour or growth habit. To illustrate from a single genus, *Penicillium purpurogenum* produces a pigment which colours the culture medium a bright purple, *P. roqueforti* is the mould used for ripening Roquefort cheese, *P. suaveolens* gives rise to a pleasant odour, whilst *P. lanosum* forms a floccose mass of mycelium which is distinctly woolly in appearance.

Many specific names, and not a few names of genera and of families, have been bestowed in honour of well-known mycologists. Other names of species are derived from place names or, in the case of parasitic fungi, from generic names of host plants. There are two ways of using proper names as names of species. The more usual way is to use the genitive case of a Latinized form of the name, as *Aspergillus Wentii* and *Penicillium Daleæ*, from Went and (Miss) Dale respectively. A less common method is to use the inflected ending -anus, -ana, -anum, as *Puccinia de Earyana* and *Heterosporium Magnusianum*. Proper names used thus are mostly written

with capital letters, although there is no uniformity as regards this practice, some authorities capitalizing personal genitives but not names derived from host genera, whilst others do not use capitals for any specific name. Proper names convey no meaning and are thus less satisfactory than descriptive names, but it is certainly very tempting to use them for new species in a genus which is already a very large one and in which nearly all the possible descriptive names have already been used, and they are undoubtedly more distinctive than a long series of binomials of colour. The custom of using generic names of host plants has unfortunately led to the reprehensible practice, not uncommon, of creating a new species every time a fresh host is found for a particular genus. Scores of species have thus been founded without any justification from adequate experimentation, with the result that some genera are hopelessly cumbered with a mass of names which ought to be relegated to the synonymy.

Family names are commonly derived from the names of the most important or most typical genera included therein. All names of families end in -aceæ (pronounced -ay'se-ee). Ordinal names, in modern usage, end in -ales (pronounced -ay'lees), but in much of the older literature the ending -ineæ is used. All names of higher rank end in -etes, pronounced as one syllable. Thus the Phycomycetes include the class Zygomycetes, one order of the latter is the Mucorales, the largest family of which is the Mucoraceæ including the genus *Mucor*. Names of classes, orders and families are capitalized but are not printed in italics. Sub-orders, sub-families, etc., are sometimes created to assist identification and rational grouping and bear names with ordinal and family endings respectively.

It is usual, when citing the name of a species, to append the name of the author who published the original diagnosis (i.e. description). The name of the authority is not written in italics. In some cases a new species is described by one worker as belonging in a certain genus and is later transferred by another author to a different genus. Both names are then given, the name of the original describer first, in brackets. For example, *Eurotium Chevalieri* Mangin is now regarded as a species of *Aspergillus* and becomes *Aspergillus Chevalieri*

(Mangin) Thom and Church. Authors' names are frequently abbreviated, or even given as initials only, as Sacc. for Saccardo, Cda. for Corda, Wr. for Wollenweber, DC. for de Candolle, B. and C. for Berkeley and Curtis, C. and E. for Cooke and Ellis.

Pronunciation

The pronunciation of names of fungi is, in this country, in accordance with the traditional English method of pronouncing Latin, with the vowels mostly as in common English words. A terminal -i is long as in "like"; a terminal -ii has the first -i short, as in "tin," and the second long; "ae" is pronounced "ee"; "g" and "c" are soft before "e," "i" and "y" and hard before other vowels, and so on. Endings such as -genum and -ferum, derived from Latin verbs, retain their original accent on the first of the two syllables. To give a few typical examples: *chrysogenum* is pronounced krī'so-jēe'num, the "y" and "e" being both long and accented; *Sphaerotheca* is sfēe'ro-thēe'ka whilst *Cephalothecium* is se'fal-o-thee'sium; fungus has a hard "g" whilst the "g" in fungi is pronounced as "j"; the "c" is hard in ascus but soft in the plural, asci. The rules given are those most commonly observed, but some mycologists modernize many names, particularly as regards accenting.

Some knowledge of Latin is essential to any student of mycology for, not only is it useful to be able to appreciate the meanings of names, but many important publications are entirely in Latin and it is now a rule, made by an International Congress of Botanists in 1930, that every description of a new species, in whatever language it is published, must be accompanied by an adequate diagnosis in Latin.

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CHAPTER III

ZYGOMYCETES

The essential features of the Zygomycetes have already been outlined in Chapter I and need not be further elaborated here. Most of the commoner forms are readily recognized as belonging to the group by their characteristic mode of growth, colonies being usually loosely floccose and of a grey or brownish grey colour. Two genera, *Mucor* and *Rhizopus*, include the great majority of the species which are usually encountered in the laboratory, but members of several other genera are found sufficiently frequently to justify their inclusion here. Zygospores have been found in comparatively few species and hence are not made the main basis of classification. However, although so many species lack the perfect fruiting stage, there has never been any question of placing these with the Fungi Imperfecti, owing to the very characteristic type of thallus which distinguishes the Class.

The Zygomycetes are divided into two orders as follows :

Asexual spores occurring in sporangia	. MUCORALES.
Asexual spores as conidia, borne on specialized conidiophores and forcibly shot away at maturity	. ENTOMOPHTHORALES.

The Entomophthorales are mentioned only for the sake of completeness since most of the species are obligate parasites on insects and only a very few have ever been grown in artificial culture. The largest and best-known genus is *Empusa*. *E. Muscæ* Cohn is of common occurrence in the late summer, when it forms characteristic white haloes round the bodies of dead flies.

Mucorales

The great majority of the species are saprophytic, occurring on a wide variety of organic substrata, whilst the remainder are parasitic on other members of the order. The typical thallus consists of coarse hyphæ growing loosely, white in the early stages of growth and becoming grey or brownish with the production of the fruiting structures. The usual mode of asexual reproduction is by spores produced in large numbers in globose sporangia, borne on special hyphæ (*sporangioophores*) which may be simple or branched in various ways. In most genera the tip of the sporangioophore is swollen, the swollen end (the *columella*) projecting into the sporangium. The columella may be of various shapes, globose, ovoid, hemispherical, etc. (Figs. 8, 9, 13), and has diagnostic importance in determination of species. The sporangial wall may be thin, when the spores are liberated by its rupture or dissolution, or may be cutinized and shot off, or broken off, in one piece. In a number of families various modifications occur of the typical globose, many-spored sporangium, the most important of these being described below.

A number of different classifications of the Mucorales have been proposed, but the total number of genera is not large and, in the great majority of cases, the identification of a fungus as far as its genus is a fairly simple matter whichever key is used for the purpose. The following simple key ignores division of the order into families and includes all the genera which are commonly found on industrial products. Many of the remainder live on dung, and although of widespread occurrence, are unable to grow on ordinary culture media.

KEY * TO THE COMMON GENERA OF MUCORALES

1. Sporangia tubular, radiating from a
vesicular swelling. *Syncephalastrum*.
- Sporangia globose or nearly so 2

* In the dichotomous system, used for this key and others later in the book (also used by many other authors), all the essential data required for identifications are arranged as *pairs* of contrasted characters, the pairs being numbered consecutively on the left. Each member of a pair leads, on the right, either to the name of a genus or to another higher number, i.e. to a further pair of contrasted features.

2. Many-spored sporangia and few-spored sporangioles present	<i>Thamnidium.</i>	
Sporangia alone produced		3
3. Sporangiphores stiff, dark coloured, metallic in appearance	<i>Phycomyces.</i>	
Sporangiphores hyaline or nearly so		4
4. Rhizoids and stolons present		5
Rhizoids and stolons absent		6
5. Sporangia globose, sporangiphores arising from points of attachment of the rhizoids	<i>Rhizopus.</i>	
Sporangia pear-shaped, sporangiphores mainly as branches from the stolons	<i>Absidia.</i>	
6. Homothallic, zygospores with very unequal suspensors	<i>Zygorhynchus.</i>	
Homo- or heterothallic, zygospores with approximately equal suspensors	<i>Mucor.</i>	

Mucor is the largest genus of the order and determination of species is far from easy. The criteria on which identifications are based are the mode of branching, if any, and the length and diameter of the sporangiphores, the colour and size of the sporangia, the character of the sporangial wall, the size and shape of the columellæ, the size and shape of the spores, the characteristics of zygospores and chlamydo-spores, if any, and general colony characteristics, such as colour and height of aerial growth. About 150 species have been described in Saccardo's Sylloge, but many of these are rare and some are of doubtful standing. Lendner (1908) describes 51 species, including the majority of those which are likely to be met with in most kinds of work. His monograph has been widely used by taxonomists and his key has been reproduced in several later publications, as for example the text-books of Harshberger and Henrici (see Chapter XIV). Since the appearance of Lendner's book Hagem (1910) has described 4 new species, Povah (1917) has added 6, and odd species have been described by a number of other workers. Until recently, however, identification of species, even with all the relevant literature available, has been difficult and often unsatisfactory. The probable reason is that in *Mucor*, as in other common

genera of moulds, certain ubiquitous species exhibit minor differences between different strains. Taxonomists who have handled comparatively small numbers of strains have not sufficiently appreciated this fact and hence have not always distinguished between true and spurious specific characteristics. Most original diagnoses have been drawn in terms insufficiently broad to cover these strain differences, with the result that workers handling *Mucors* have but rarely found strains which tallied exactly with published descriptions.

In a recent work on the Mucorales, Zycha (1935) has considerably simplified the taxonomy of *Mucor* by taking into account this natural variation. The total number of species has been reduced to 42 and, of these, several, of which type cultures are not available, are accepted only provisionally. Zycha's monograph is easily the most satisfactory treatment up to date both of this genus and of the remaining genera of Mucorales, and seems likely to come into general use by taxonomists.

M. mucedo Linné is the type species of the genus, having first been described in 1762. It is found chiefly on dung, where it forms erect sporangiophores up to 15 cm. long, with greyish sporangia encrusted with crystals of calcium oxalate. In culture the sporangiophores are usually shorter and bear at the base short branches terminating in small sporangia. The terminal sporangia are 100–200 μ in diameter, with diffuent wall; columellæ pear-shaped to cylindrical, often with orange-coloured contents; spores elliptical, 6–12 μ long.

M. piriformis Fischer is of fairly common occurrence in any kind of damp situation. It forms dense, cottony, grey colonies, 2–3 cm. high, with sporangiophores mostly unbranched, up to 50 μ in diameter. Sporangia are globose and very large, 250–350 μ diam., with diffuent wall. The name is derived from the shape of the columellæ, which are mostly definitely pear-shaped, 200–300 μ long. The spores are elliptical, 5–13 μ by 4–8 μ .

M. racemosus Fresenius is probably the most widely distributed of all the *Mucors*, and has frequently been redescribed under other names. It is found on almost every kind of damp material. Colonies are grey or brownish grey, of loose texture and normally less than 1 cm. high. The sporangio-

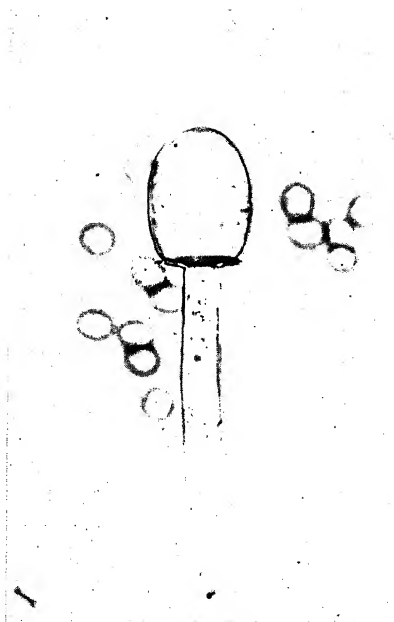


FIG. 8.—Oval columella with collar-ette—*Mucor racemosus*. $\times 500$.

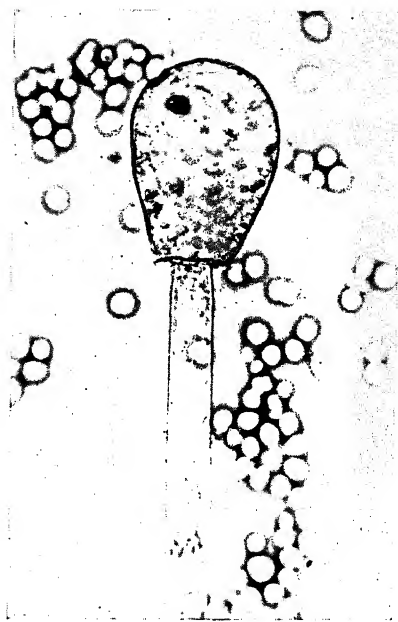


FIG. 9.—Pyriform columella with small collar-ette. $\times 500$.

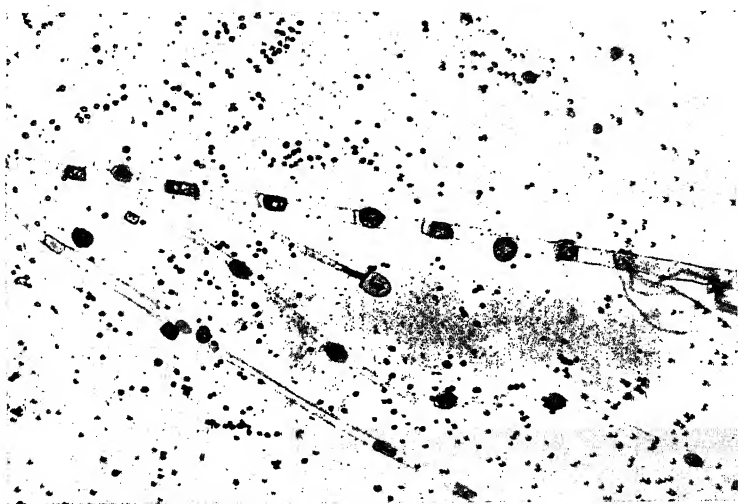


FIG. 10.—Chlamydospores in sporangiophore of *M. racemosus*. $\times 100$.



FIG. 11.—Sporangiophore of *M. spinosus* showing cymore branching, as seen in living culture (tube). $\times 50$.

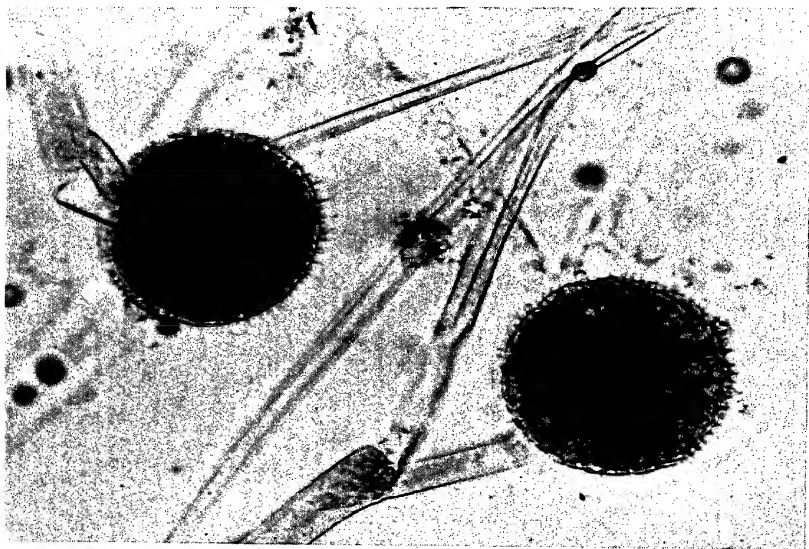


FIG. 12.—*M. spinosus*—young sporangia showing spinescent walls. $\times 500$.

phores are simple at first, later becoming branched, with the branches irregularly placed and very unequal in length. Sporangia are globose, very unequal in size but mostly small, $20\text{--}70\mu$ diam., with walls which break in pieces when handled. The columellæ are spheric or oval with collarette (a portion of the broken sporangial wall left *in situ*) (Fig. 8), and the spores are mostly elliptical, $6\text{--}10\mu$ by $5\text{--}8\mu$. The most characteristic feature of the species is the abundant production of chlamydospores, which are formed in aerial hyphæ, along the sporangiophores and even in the columellæ. They are of diverse shapes, colourless or yellow, smooth, and about 20μ in diameter (Fig. 10). *M. racemosus* can grow completely submerged in liquid media containing sugar, when it forms chains of yeast-like cells and, like yeast, produces alcohol.

M. spinosus van Tieghem (synonyms *M. spinescens* Lendner and *M. plumbeus* Bonorden) is of very common occurrence. Colonies are at first white, then dull grey and finally brownish grey, only a few millimetres high. Sporangiophores show sympodial branching (see Fig. 11) and are about 1 mm. long and 10μ diam. Sporangia are nearly spherical, usually slightly flattened, very even in size, about 65μ diam., and are encrusted with fine needle-shaped crystals, thus appearing spinescent (Fig. 12). The columellæ are very characteristic, being oval or pyriform with curious spiny projections at the top (Fig. 13). The spores are round, $7\text{--}8\mu$ diam., occasionally somewhat smaller.

M. Rouxii (Calmette) Wehmer was originally isolated from Chinese rice by Calmette and named *Amylomyces Rouxii*. It was the first of a series of species of the Mucoraceæ to be used for the manufacture of alcohol by the "Amylo" process (Calmette, 1892; Collette and Boidin, 1897, 1898). It grows, but does not usually form sporangia, on most ordinary media. On rice it grows normally, colonies being reddish in colour, with short, sparingly branched sporangiophores, small sporangia $20\text{--}30\mu$ diam., elliptical spores about 5μ long, and abundant chlamydospores, yellow to brown and up to 100μ in diameter.

M. hiemalis Wehmer is a common soil organism and hence is found on numerous soil-contaminated products. Colonies are yellowish or clear grey, 1–2 cm. high; sporangiophores

simple or sympodially branched; sporangia $50\text{--}80\mu$ diam., olive to greyish brown when ripe, with diffuent wall; columellæ spheric or oval with collarette, up to 50μ long; spores irregular in shape but mostly roughly oval, $3\text{--}9\mu$ by $2.5\text{--}5\mu$; chlamydospores formed in mycelium. The mould is heterothallic and both $+$ and $-$ strains are known.

M. Ramannianus Möller is another fairly common soil organism. Colonies are at first pinkish to brownish red, turning grey, only about 1 mm. high; sporangiophores mostly unbranched, $2\text{--}6\mu$ diam.; sporangia reddish, small, $20\text{--}40\mu$ diam., with diffuent wall; columellæ spheric, $5\text{--}10\mu$ diam.; spores globose to short oval, $2\text{--}3\mu$ long; chlamydospores numerous and of various shapes; giant cells formed in submerged mycelium.

Zygorhynchus is a small genus of moulds mostly inhabiting the soil. The genus was not recognized by Lendner but is separated from *Mucor* by most other authorities. All the species are homothallic and differ from *Mucor* in that the suspensors are very markedly unequal in size (Fig. 14). Two species are of common occurrence in this country.

Z. Moelleri Vuillemin grows well on all media, forming a loose felt only a few millimetres high. Abundant zygospores are formed within two to three days, sporangiophores being more sparingly produced somewhat later. The sporangiophores are simple or irregularly branched, bearing grey sporangia which are mostly slightly broader than long, usually 48 by 50μ . Columellæ are definitely obovate, averaging 25 by 30μ , and the spores are oval, $4\text{--}5\mu$ by 3μ . The zygospores are formed on bifurcated hyphæ, are spherical, $35\text{--}50\mu$, dark brown to black, and covered with short thorn-like projections.

Z. heterogamus Vuillemin is somewhat similar to the last species. Sporangiophores are branched, often more or less verticillate with two to four branches, sporangia spherical $50\text{--}60\mu$ in diameter, columellæ spherical, and the spores round, $2\text{--}3\mu$ in diameter. Zygospores are black, verrucose, varying in size from 45 to 150μ . Chlamydospores are formed in the mycelium.

Rizophus. Species of this genus occur on all kinds of material and are common as aerial contaminants in the laboratory. On most culture media they grow with extreme rapidity, spreading widely by means of their stolons. They completely

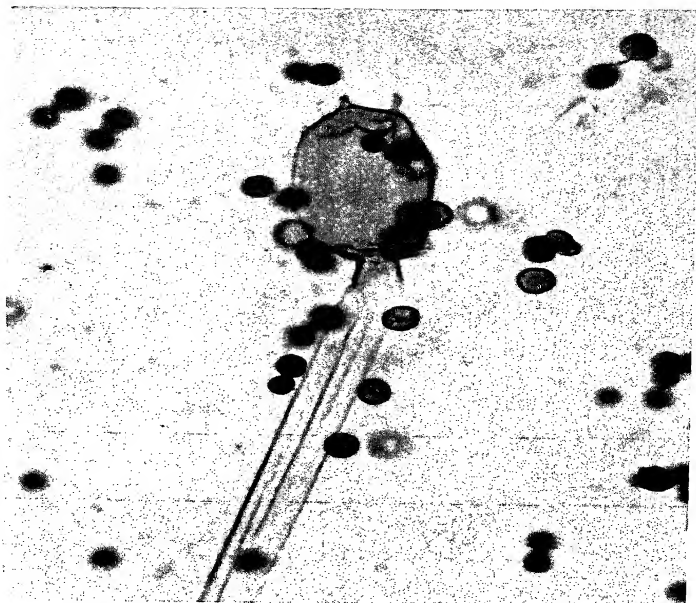


FIG. 13.—*M. spinosus*—columella with terminal spines. $\times 500$.

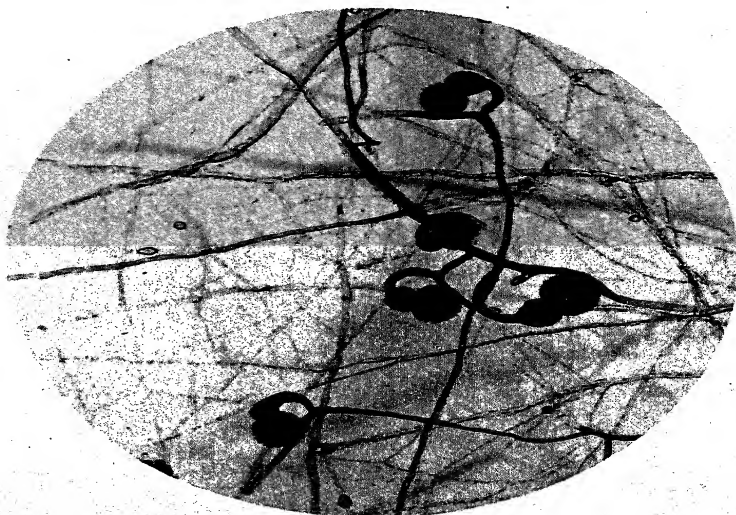


FIG. 14.—*Zygorhynchus Møelleri*—zygospores (from tube culture). $\times 100$.

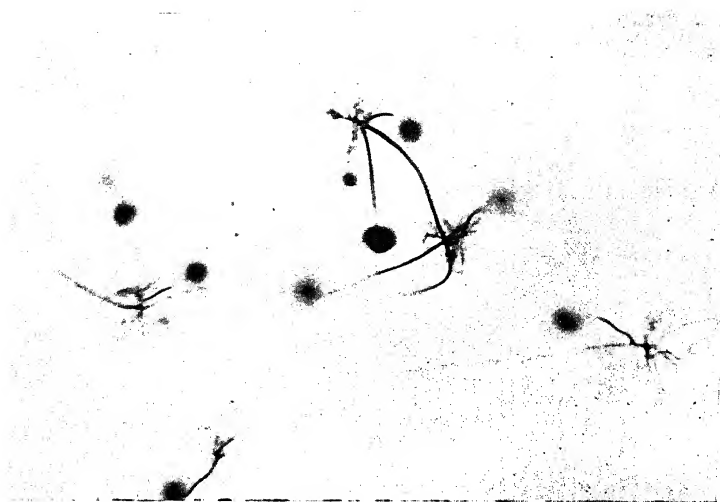


FIG. 15.—*Rhizopus nigricans* showing rhizoids and stolons (Petri dish culture). $\times 50$.

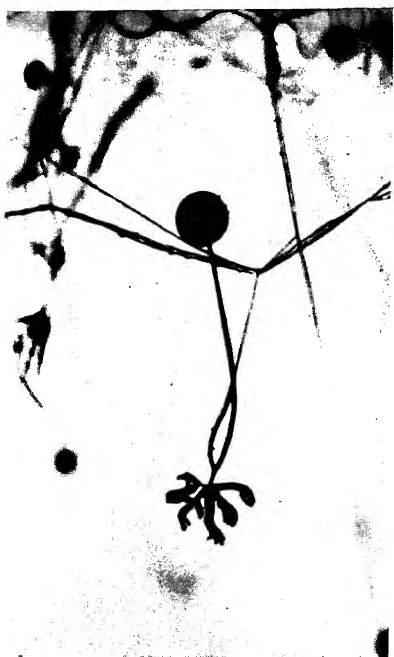


FIG. 16.—*Rh. nigricans*—rhizoids and sporangium (tube culture). $\times 50$.



FIG. 17.—*Absidia spinosa*—formation of zygospores showing outgrowths from one suspensor. $\times 250$.

fill culture tubes and Petri dishes with dense cottony masses of mycelium, and can be a great nuisance from their habit of sporang along the line where the cover touches the edge of the dish, thus shedding spores outside. The *Rhizopi* are readily distinguished from *Mucors* by the presence of stolons (runners), often several centimetres long, and of tufts of root-like hyphae (rhizoids) emerging from the points where the stolons touch the medium or the surface of the glass, also by their greyer colour and greater luxuriance of growth. Lendner (1908) describes 22 species, but some of these are rare, and a number of others are, except for the specialist, best regarded as strains of *R. nigricans*. *R. nigricans* Ehrenberg is of world-wide distribution and is found on all kinds of mouldy material. It is frequently the first mould to appear on stale bread and is often found on other food-stuffs in the home. Colonies spread very rapidly, completely filling tubes and Petri dishes in two to three days. Stolons are clearly differentiated, arising from and terminating in strong tufts of dark brown rhizoids (Figs. 15, 16). The sporangiophores are erect, arising in groups opposite the rhizoids, up to 2.5 mm. long and about 20μ in diameter, bearing black, globose sporangia, up to 200μ in diameter. The spores are variously shaped, ovate, polygonal or angular, striated, and mostly $6-9\mu$ in long axis.

R. arrhizus Fischer is distinguished from *R. nigricans* by its somewhat less rampant growth and by the character of the rhizoids, which are pale in colour, short and ragged.

Two other species of *Rhizopus*, *R. japonicus* Vuillemin and *R. tonkinensis* Vuill., both of oriental origin, are of note because they successively replaced *Mucor Rouxii* for the saccharification of starch in the Amylo process of manufacturing alcohol. They are of no importance in other connections and are not likely to be met with in ordinary mycological work.

Absidia. Species of this genus differ in several respects from the *Rhizopi*. The sporangiophores arise from the internodes of the stolons rather than from the points of attachment of the rhizoids, the sporangia are pear-shaped and the zygo-spores are surrounded by coarse, hairy outgrowths from the suspensors. The latter feature is highly characteristic and suffices to place at once the homothallic species of the genus (Fig. 17). Several species have been described, but none of

them are of such common occurrence as species of *Mucor* and *Rhizopus* and they are of little technical importance.

Phycomyces. Several species are known, but only one is commonly encountered. *Ph. nitens* (Agardh) Kunze occurs mainly on fatty products and particularly in empty oil casks. It grows well on wort agar and some media made from plant extracts, but poorly on synthetic media. The sporangiophores are stiff, almost like wire, dark coloured and with a pronounced metallic sheen. In test-tube cultures they curl round in order to accommodate their length to the confines of the tube, but in the natural habitat of the species they are approximately straight and up to about 30 cm. in length. *Ph. nitens* is positively phototropic and, in the laboratory, can be induced to form its characteristic long sporangiophores by growing on a rich medium, such as wort, contained at the bottom of a tall vessel, the sides of the latter being covered with some opaque material so that light can enter only from the top.

Thamnidium differs from the preceding genera in that the sporangiophores bear lateral clusters of sporangioles as well as large terminal sporangia. The sporangioles resemble miniature sporangia, containing from two to a dozen or more spores, and are formed on richly branched outgrowths from near the base of the sporangiophore (Figs. 18, 19, 20).

Th. elegans Link is a fairly common mould and grows well on most culture media. It rapidly forms a granular, greyish growth a few millimetres high, consisting chiefly of sporangioles. Later the sporangiophores extend to a length of 1 cm. or more before forming the large globose terminal sporangia, which are dark brown in colour and 150–250 μ in diameter. The spores in the sporangioles are indistinguishable from those in the main sporangia. They are ovate or bean-shaped, 8–12 μ in long axis.

Syncephalastrum is a small genus of tropical moulds, but one species, *S. cinereum* Guéguen, is fairly often found on raw materials brought into this country. It grows luxuriantly in the laboratory, forming colonies resembling in texture those of a *Rhizopus*, with black heads produced on short branches from trailing hyphæ. When mounted and viewed at a low magnification the heads bear a striking resemblance to heads of *Aspergillus* (Fig. 21). At a higher magnification the chains



FIG. 18.—*Thamnidium elegans*
—young sporangiophore
with clusters of sporangia
(Petri dish culture). $\times 50$.

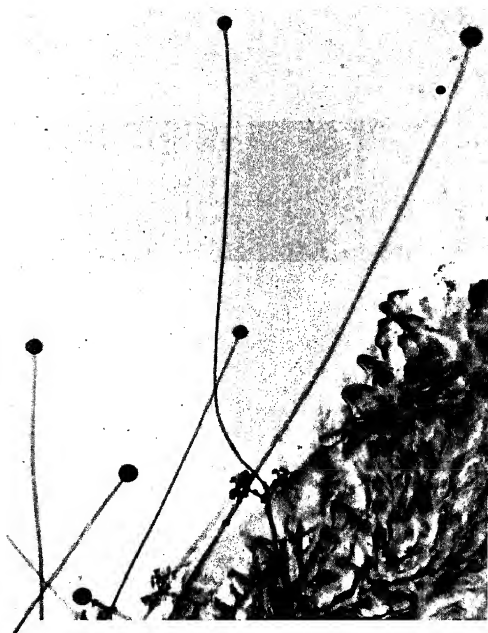


FIG. 19.—*Th. elegans*—mature sporangiophores
with terminal sporangia (from culture on
film of agar between two glass plates).
 $\times 25$.



FIG. 20.—*Th. elegans*—sporangiole

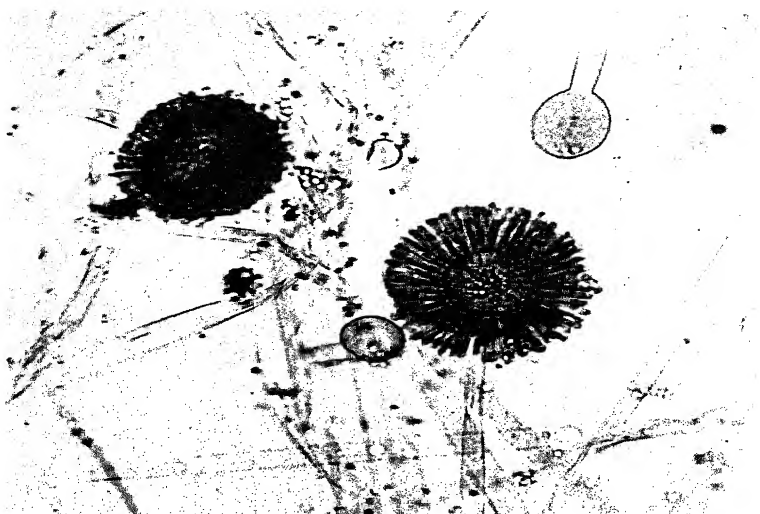


FIG. 21. *Syncephalastrum cinereum*—spore heads.
 × 250. Note the resemblance to *Aspergillus*.

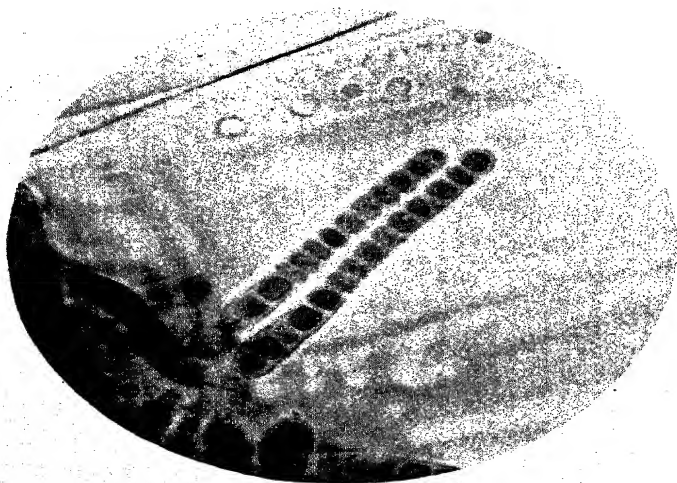


FIG. 22.—*S. cinereum*—tubular sporangia, × 1000.

of spores are seen to be contained in long tubular sporangia which radiate from the swollen end of the sporangiophore. Fig. 22, taken at a high magnification, shows clearly that the spores are formed within, and distinct from the sporangial wall.

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CHAPTER IV

THE ASCOMYCETES

The Ascomycetes constitute the largest of the classes of perfect Fungi, the number of species known being approximately 15,000. As may be expected in such a large group there is considerable diversity of form and structure. At one end of the scale are the unicellular organisms commonly known as yeasts, and, at the other, species with extensive mycelium and large and elaborate fruiting structures, such as the truffles.

The *ascus*, a structure which is peculiar to and which gives the name to the group, is a thin-walled receptacle enclosing the spores and usually rupturing at maturity. The ascus is distinguished from the sporangium of the Phycomycetes in the origin and method of formation of the spores, details of which are to be found in any work on Systematic Mycology. A more obvious difference is that the sporangium contains an indefinite number of spores, whilst in the great majority of Ascomycetes the ascus invariably contains 8 spores. In a few cases, which are of no importance here, the regular number is a multiple of 8, or, more strictly, a higher multiple of 2. In a few primitive members of the class, notably in *Endomyces*, the number is 4, whilst in the yeasts the number ranges from 1 to 8. In the simpler Ascomycetes the ascus is more or less globose, with the spores packed together, whilst the *ascocarp*, i.e. the fruit-body containing the asci, is globose with the asci arranged irregularly or entirely lacking, the asci then being produced either singly or in loose clusters. In the higher members the ascocarp takes definite form, with club-shaped asci arranged in parallel series, often with elongated sterile cells (paraphyses) separating them. If the ascocarp is globose or flask-shaped and closed at maturity it is called a *peri-*

thecium, if wide open at maturity an *apothecium*. The primary classification of the Ascomycetes is based on the form of the ascocarp. Many different classifications have been proposed, probably the most satisfactory being that of Gwynne-Vaughan and Barnes (1937).

Ascocarp, if present, either with no definite ostiole, or shield-shaped, or with asci irregularly arranged	PLECTOMYCETES.
Ascocarp wide open at maturity, asci in parallel series	DISCOMYCETES.
Ascocarp flask-shaped when ripe, asci in parallel series	PYRENOAMYCETES.

The Discomycetes include about 4000 species, very many of them parasitic, but some saprophytic in the soil or on dung. A number of the larger forms are fleshy and a few, such as the truffles and morels, edible.

The Pyrenomycetes form the largest of the three groups, with about 10,000 species. Many common and serious plant diseases are caused by members of this group, but there are also a large number of saprophytic species and a very few which may be classed as moulds. The latter will be discussed below.

The Plectomycetes are divided into three orders, Plectascales, Erysiphales and Exoascales, of which the two latter include only obligate parasites and have asci arranged in parallel series like the Pyrenomycetes. The Plectascales include the great majority of the Ascomycetes which are of industrial importance.

The Yeasts

The fungi known as yeasts are organisms of the simplest possible structure, consisting of single cells, which may be globose, ovoid, pyriform or more or less elongated, reproducing by budding and mostly detached from each other, only forming loose associations somewhat resembling mycelium when growing very vigorously. Certain species are used for the venerable processes of baking and the production of alcoholic beverages by fermentation, for they secrete enzymes which convert sugar into alcohol and carbon dioxide. Others are encountered as contaminants in fermentation processes, where their presence is undesirable on account of their production of unpleasant flavours. Some of the yeasts, and also a number

of closely allied imperfect fungi usually classed as *Torulaceæ*, are common accompaniments of moulds, frequently appearing in large numbers when mouldy products are plated out. They are readily recognized as they form moist colonies, somewhat slimy in texture, usually whitish, cream or pink, but occasionally otherwise coloured, and, when mounted in fluid usually show under the microscope all stages of the characteristic budding process. When about to reproduce, the mature cell puts out a nipple-like projection; as this grows a constriction develops between the future daughter cell and the parent; after a period of further growth, in which the projection rounds up to approximately the same shape as the parent cell, the constriction deepens and the new cell becomes detached.

Under certain conditions, which cannot be exactly defined for any one species, much less for the whole family, but which usually involve a sudden check to a vigorously growing culture, any of the cells may develop into an ascus. Since the latter is an envelope enclosing one or more spores, each spore with its own definite wall, it is readily distinguished from a vegetative cell.

The unicellular organisms which reproduce by budding, and are often collectively referred to as yeasts, are divided into two main groups, the yeasts proper, the *Saccharomycetaceæ*, including only species known to form ascospores, and the *Torulaceæ* or non-sporing yeasts. The latter are strictly *Fungi Imperfecti* but are conveniently considered along with the true yeasts.

The family of *Saccharomycetaceæ* includes, according to the latest authorities, 17 genera, distinguished according to the mode of formation of the ascus and the number of spores therein. Recognition of genera and species is difficult, since sporing is often uncertain and some species, after cultivation for some time, seem to lose altogether the power of producing ascospores. Perhaps fortunately, the majority of the genera are of academic interest only, all the species of industrial importance belonging to the genus *Saccharomyces*. The whole family is treated fully in the monographs of Guilliermond (1912, 1920) and Stelling-Dekker (1931), and these should be consulted by those who are interested in the more theoretical aspect of Mycology.

Saccharomyces. The genus includes a large number of species which, largely on account of the unicellular nature of the organisms, cannot be completely characterized on morphological data alone. Physiological characteristics, in particular the varied power of fermenting different sugars, are used, along with such data as size and shape of cells, texture of colonies on various media and rates of growth, in defining species.

S. cerevisiæ Hansen is widely known and used as brewers' and bakers' yeasts. The normal cells vary considerably in size, the usual range being $1-5(9)\mu$ by $1-4(5)\mu$, and appear roughly circular when viewed in optical section. Actually, in this as in other species, the shape varies somewhat widely, ranging from round to elongated, but a fair proportion of the cells are circular and the average shape is more nearly circular than in other species. A number of strains with trade names have been regularly propagated since the introduction into the brewing industry of pure culture methods. There are two main groups of these, known respectively as "top yeasts" and "bottom yeasts." The top yeasts produce CO_2 vigorously and the cells have a tendency to become attached in clusters. They are carried to the surface of the liquid during the fermentation, by the rising gas, and there form a scum. Top yeasts are used for brewing the typically English beers and ales. Bottom yeasts are used for the preparation of the light Continental beers typified by the well-known "lager." In these a prolonged fermentation is carried out at a comparatively low temperature and the yeast cells stay at the bottom of the liquor. Distillers use top yeasts and carry out the fermentation at a high temperature in order to obtain rapid production of alcohol in maximum yields. Bakers' yeast is largely produced as a side line by the distillers, the mass of cells produced during the fermentation being separated by centrifuging, washed and pressed for marketing. Brewery yeast is less suitable for baking since it is rendered bitter and dark in colour by the hops added to the wort before fermentation.

S. ellipsoideus Hansen. As the name implies the cells are, on the average, more oval in section than those of *S. cerevisiæ*. It is the yeast used for the fermentation of wine, its occurrence on grapes and in the soil of vineyards accounting for the success of vinous fermentation during countless ages before the

nature of the process was understood. At the present day pure culture methods are used to some extent in the wine industry and, as in the case of *S. cerevisiæ*, a number of strains with special trade names are in regular use for the production of different classes of wine.

S. pastorianus Hansen is best known for its production of unpleasant flavours in beer, in which it occurs as a contaminant. It is distinguished by the characteristic shape of the cells, usually described as sausage-shaped.

S. fragilis Jörgensen (syn. *S. Kefir*) is used for the preparation of fermented liquors from milk, such as Kefir and Koumyss. It is a bottom yeast capable of fermenting lactose and producing therefrom much CO₂ and comparatively little alcohol. In the countries where such milk products are made, pure culture methods are not used and the actual agent of fermentation is usually a mixture of various yeasts and bacteria, in which however *S. fragilis* predominates.

Other species and strains of the genus *Saccharomyces* are responsible for the alcoholic fermentation of cider and perry, the wild yeasts occurring along with the respective raw materials being relied on almost exclusively. In the East, alcoholic liquors are made chiefly from rice. Yeasts commonly take some part in the fermentation, but the main agents are moulds.

Pichia and *Willia* are two genera known best as contaminants in the brewing industry. They produce dry, wrinkled pellicles on liquid media and produce no alcohol but small quantities of esters. They can actually utilize alcohol as a source of carbon and hence can constitute a very serious nuisance.

Torulaceæ

The name *Torula* was first used by Persoon in 1801 for a genus of fungi belonging to the Dematiaceæ. Since the time of Pasteur, however, workers in the fermentation industries have applied the name to the non-sporing yeasts, and, in spite of the laws of priority, both types of organism continue to be called by the same name. Will (1916) first proposed to raise the group of non-sporing yeasts to the rank of family, the Torulaceæ, and this suggestion has been followed by a number of subsequent authors. A useful treatment of the family is by Harrison (1928) who divides it into four genera as follows :

Producing red pigment	<i>Rhodotorula</i> , with 13 species.
Producing pigments other than red	<i>Chromotorula</i> , with 4 species.
No pigment produced	
Hyphæ formed	<i>Mycotorula</i> , with 10 species.
No hyphæ formed	<i>Torula</i> , with 16 species.

The species are distinguished according to their powers of fermenting various substrata as well as by morphological characteristics. The mass of data given by Harrison cannot be briefly summarized and the original paper should be consulted for details.

More recently Lodder (1934), in the first part of a monograph on the non-ascosporic yeasts, recognizes two families, with a provisional third family comprising a single doubtful genus. The Rhodotorulaceæ, with a single genus *Rhodotorula*, includes all species which produce carotinoid pigments and covers Harrison's two genera *Rhodotorula* and *Chromotorula*. The Torulopsidaceæ includes all species without carotinoid pigments, and is divided into two sub-families. The first, Torulopsoideæ, corresponding roughly to Harrison's genus *Torula*, comprises 7 genera with 43 species and 3 varieties. Treatment of the second sub-family, Mycotoruloideæ, is reserved for the promised second half of the monograph. It is of interest to note that Lodder adopts a suggestion, first proposed by Berlese, to end the confusion in nomenclature by reserving the name *Torula* for Persoon's genus and using the name *Torulopsis* for a genus of non-sporing yeasts.

Other Ascomycetes

Endomyces, a genus containing very few species, none of them common, is closely related to the yeasts. It is distinguished by its formation of definite, though scanty, mycelium, bearing solitary globose asci, each containing 4 spores. Its chief interest is to the systematist as a link between the true yeasts and the higher Ascomycetes. One species, *E. vernalis* Ludwig, has been used for the large-scale production of fat from carbohydrates (see Chapter XIII).*

Byssochlamys is also of interest to the systematic mycologist as it produces clusters of 8-spored asci without any surrounding wall (peridium) and thus forms a link between the Endomycetaceæ, with solitary 4-spored asci, and the Gymnascaceæ and

Aspergillaceæ with more or less definite perithecia containing 8-spored asci. In addition, *B. fulva* Olliver and Smith (1933) has assumed considerable importance in recent years as a cause of spoilage in canned and bottled fruits. The mature ascospores can withstand a much higher temperature than can the spores of most fungi and sometimes survive the commercial sterilizing process. The fungus has also the power of growing in an atmosphere containing very little oxygen, or even completely submerged in liquid, where the only oxygen available is the small amount dissolved. Spoiled fruit frequently shows no visible colonies of the mould and the cans are not blown as when the spoilage is due to anaerobic bacteria, the only evidence of the presence of the fungus being a general softening, or sometimes complete disintegration, of the fruit, such as would occur if cooking had been unduly prolonged.

The mould grows well on all ordinary media, with colonies of loose cottony texture, becoming fulvous (tan to sandy brown) as spores develop. The conidial fructification is of the *Pæcilomyces* type (see Chapter VIII) with long chains of ovate spores, $4-9\mu$ by $2.3-2.5\mu$ (Fig. 23). The asci develop rapidly in compact clusters, without any trace of peridium (Fig. 24), each ascus containing 8 ovate spores; $6-6.5\mu$ by $4.3-4.5\mu$. *B. nivea* Westling is similar, but the colonies are white, only turning slightly brown in age, and the ascospores are somewhat smaller than those of *B. fulva*.

Eurotium is the name given to the perfect stage of the fungus, or rather group of fungi, more commonly known as *Aspergillus glaucus*. The conidial and ascospore phases were long thought to be separate and distinct species until De Bary in 1854 showed their real relationship. Further details are given in the chapter on *Aspergillus* (Chapter VII).

Monascus. *M. purpureus* Went is not uncommon, particularly in dairy products, and is the organism which gives the characteristic colour to Chinese red rice. The perithecia are produced singly on stalks (Fig. 25), whilst the ovate conidia are formed in short chains (Fig. 26). The generic name was bestowed at a time when the perithecium was thought to constitute a single, many-spored ascus. It is now known that normal asci are produced, but these break up rapidly at maturity, leaving the somewhat thin-walled perithecium full of

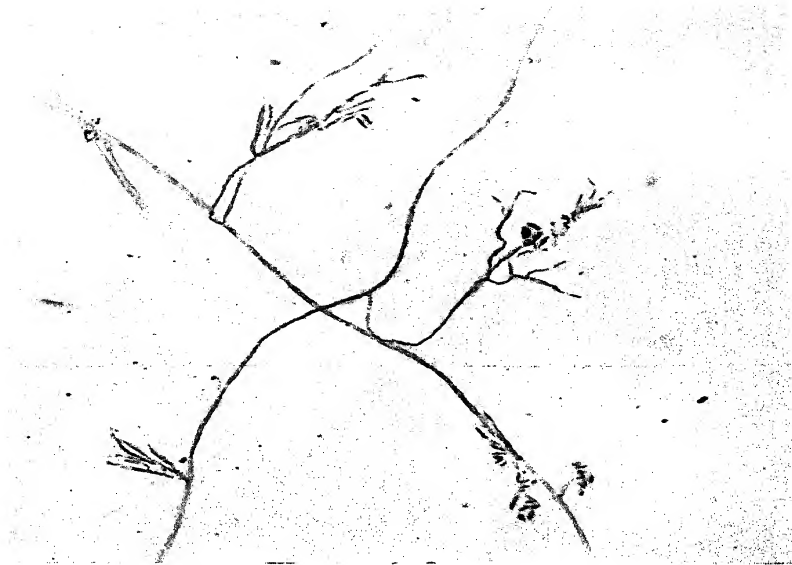


FIG. 23.—*Byssoschlamys fulva*—conidiophores (from slide culture). $\times 250$.

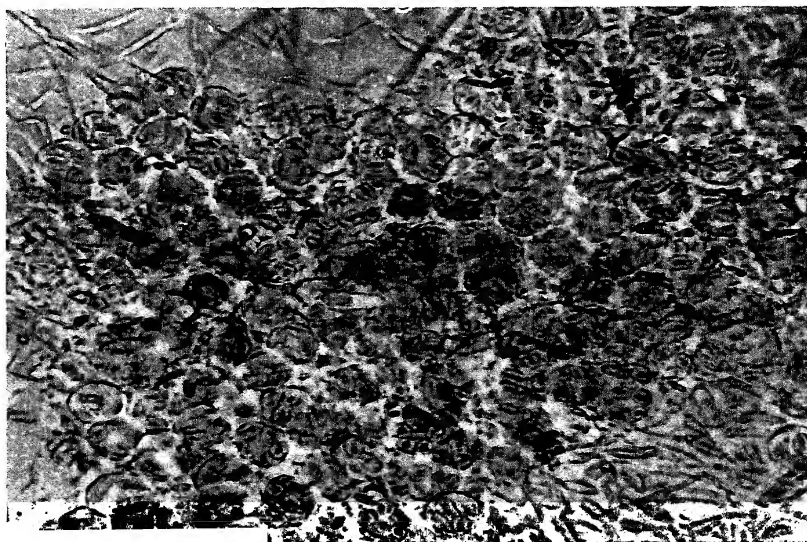


FIG. 24.—*B. fulva*—cluster of asci. $\times 500$.

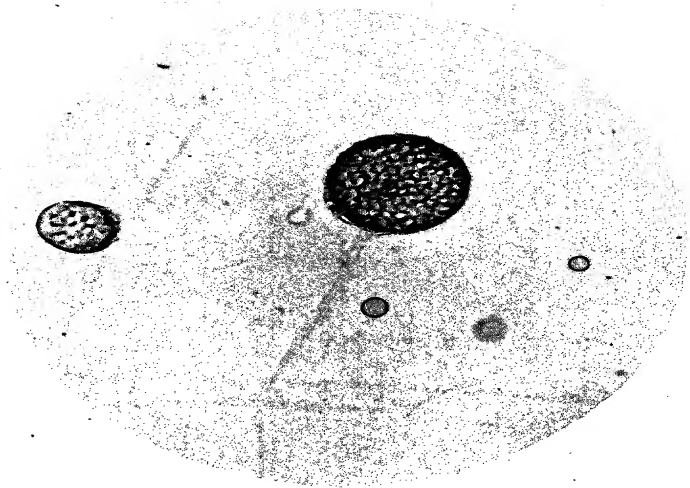


FIG. 25.—*Monascus purpureus*—stalked perithecium.
× 250.



FIG. 26.—*M. purpureus*—young perithecium and
conidia. × 250.

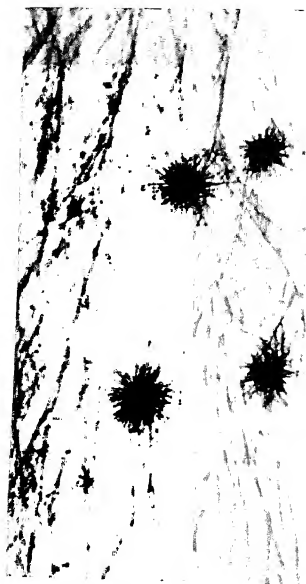


FIG. 27.—*Chaetomium globosum*—perithecia in living culture. $\times 25$.

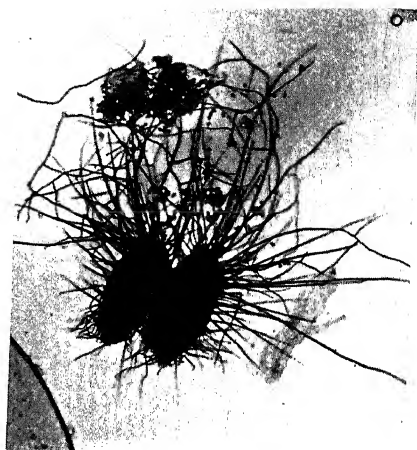


FIG. 28.—*Chaetomium chartarum*—perithecia. $\times 60$.

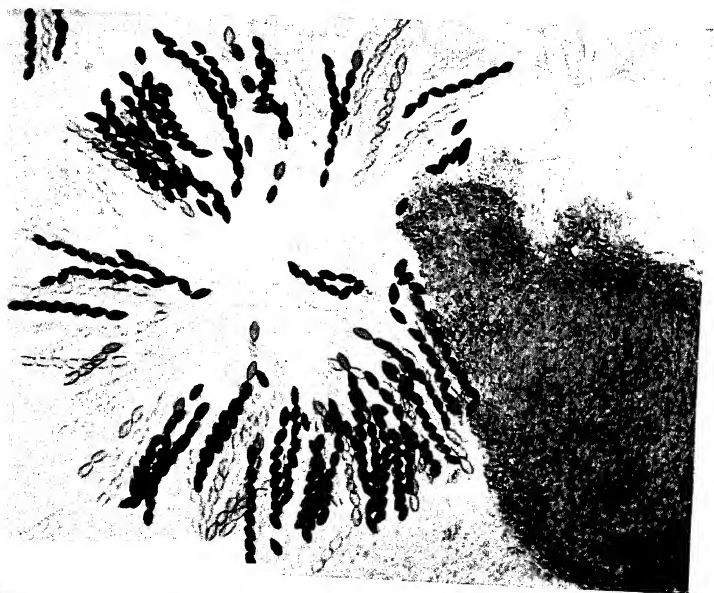


FIG. 29.—*Neurospora sitophila*—crushed perithecium. $\times 100$.

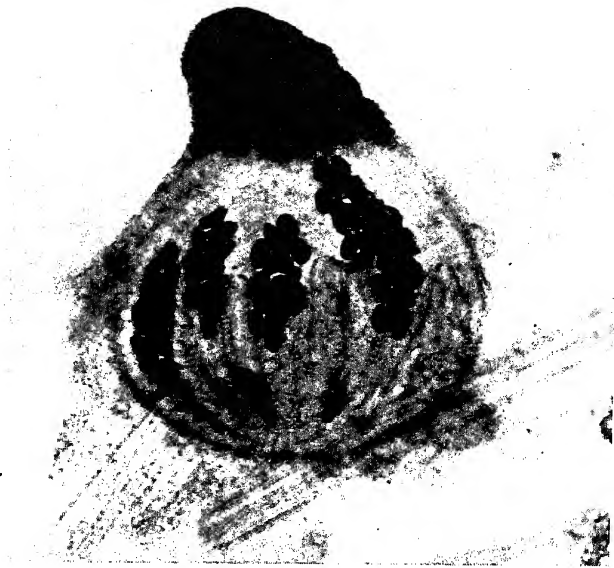


FIG. 30.—*Sordaria* *sp.*—perithecium in optical section. $\times 100$.



FIG. 31.—Typical Pyrenomycete (unidentified)—crushed perithecium. $\times 100$.

loose spores. Colonies form a rather thin, spreading growth, of a pronounced reddish or purple colour.

Chaetomium is probably the only genus of Pyrenomycetes at all commonly encountered in industrial work. A number of species are known, all characterized by black perithecia with short necks and beset with long stiff hairs, which are variously straight, branched or curled. They are found chiefly on cellulosic materials and thrive particularly on paper. When kept in culture a strip of filter paper, only partly immersed in an ordinary culture medium, usually ensures satisfactory production of perithecia. *C. globosum* Kunze grows vigorously in culture, the colonies being brownish yellow, the perithecial hairs coiled at the ends (Fig. 27) and the spores brownish, elliptical with more or less pointed ends, in size about $9 \times 7\mu$. *C. chartarum* Berk. has brownish mycelium, perithecial hairs dichotomously branched and brownish spores elliptical in section (Fig. 28).

Neurospora is the name given to the perfect stage of the red bread-mould, *Monilia sitophila* (Montagne) Saccardo. Since the fungus is heterothallic perithecia are obtained only when the mycelia of two strains of opposite sex come in contact (Shear and Dodge, 1927). (See Fig. 29.)

A number of other Ascomycetes, mostly plant parasites or saprophytes on plant residues, strayed from their natural habitat, are occasionally encountered in mixed cultures. Many of them are sterile under ordinary laboratory conditions and cannot be recognized unless previously obtained from a known source, but a few, such as *Sordaria* and *Pleospora*, frequently grow well and produce normal perithecia (Fig. 30). Dark coloured perithecia with walls which are opaque under the microscope can be distinguished from pycnidia of similar gross appearance by gently crushing them under a cover-glass, when it is nearly always possible to find immature asci containing the normal 8 spores (Fig. 31). Such species are of no particular importance outside the province of plant pathology.

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CHAPTER V

FUNGI IMPERFECTI

As already stated in Chapter I, the class usually known as the Fungi Imperfecti (Deuteromycetes of some authors) includes all those species of Mycomycetes which have no perfect fruiting stage but reproduce solely by means of asexual spores or by fragmentation of vegetative structures. In addition, it is common practice to include amongst the Fungi Imperfecti a number of species which are known to be merely the conidial stages of perfect fungi, mostly of Ascomycetes. Many *Fusaria*, for example, are imperfect stages of *Nectria*, *Calonectria*, *Gibberella* and *Hypomyces*; *Cladosporium herbarum* is reported to be the conidial form of *Microsphaerella Tulasnei*; *Aspergillus glaucus* belongs with *Eurotium*; several species of *Penicillium* produce ascospores and there are numerous other similar connections known. From time to time fresh cases are brought to light and it is extremely probable that a large number of the Fungi Imperfecti have life cycles which are incompletely known rather than incomplete. A few purely conidial forms have been proved to be haplont strains of heterothallic species—*Monilia sitophila* and related species are examples—and it may be that there are many more similar cases as yet undiscovered, awaiting some chance observations, or systematic experiment on a large scale, for their elucidation.

Strictly speaking, the discovery of the perfect stage of a fungus, or of the connection of an imperfect form with an already known perfect species, ought to result in the separate name of the conidial form being dropped. The retention of such names, and the inclusion of the species in the Fungi Imperfecti, is purely a matter of convenience. Classification is intended to facilitate identifications just as much as it is designed to exhibit

natural relationships. Many species, of which the perfect stages are known, form asci only under very special conditions of culture or only when the fungi are parasitic, conidia alone being produced in ordinary laboratory cultures. Such species would be very difficult to identify if they had to be sought in a classification based on characteristics of sexual spores and fruit-bodies, and they are best left in the Fungi Imperfecti. There are even a few species, such as *Aspergillus glaucus*, which are conveniently classified along with forms of similar conidial morphology in spite of the fact that perithecia are formed readily and abundantly under almost any conditions of culture.

It should be understood that the terms "family" and "genus" have not the same significance in the Fungi Imperfecti as in the Ascomycetes and Basidiomycetes. Species with similar cultural characteristics and conidial fructifications of the same type, and which are therefore classed together, may produce perithecia of quite different types, and hence belong in different genera of Ascomycetes. *Aspergillus glaucus* (= *Eurotium herbarum*), for example, has soft, yellow perithecia, whilst *A. nidulans* has perithecia which are dark coloured and brittle, and has been made the type of the genus *Diplostephanus* by Langeron. Three different types of perithecia have been described for species of *Penicillium*, and, in a rational classification, these species would be placed in three different genera. Conidial forms known as *Eotrytis* are known to belong to several different genera of Ascomycetes and the same thing obtains, as stated above, for species of *Fusarium*. In addition, it is not unusual for the species in a single genus of Ascomycetes to have conidial stages which are sufficiently varied to be classed in different genera of Fungi Imperfecti. On account of these facts many mycologists use the term "form-genus" for a group of imperfect species with similar conidial fructifications, that is for what is here termed a "genus" of the Fungi Imperfecti. Moreover, it is by no means rare for a single species to produce more than one kind of asexual spores in response to varying conditions, and the different stages of such fungi have been regarded as distinct species until such time as the true life histories have been worked out. Arguing from the probability that many Fungi Imperfecti which have been incompletely studied are similarly related one to another, the term "form-



FIG. 32.—*Sphæropsidale* sp.—crushed pycnidium. $\times 100$.

species " has been used by some workers. In this and the following chapters the terms "genus" and "species" are used without qualification, but they are not to be regarded as having the same significance as when applied to perfect fungi.

The number of Fungi Imperfecti is very large, exceeding that of the Ascomycetes and, therefore, the construction of a satisfactory scheme of classification is a matter of great difficulty. The system in general use is that of Saccardo (1884). It is the only system which has been fully worked out and which includes all known genera. From several points of view it is irrational, but it is usually workable and, until someone evolves a better scheme equally complete, it is likely to retain general acceptance.

The Fungi Imperfecti are divided into three orders, as follows :
(In some cases Saccardo's terminology is modernized)

Conidiophores produced inside flask-shaped receptacles (pycnidia)	SPHÆROPSIDALES.
Conidiophores occurring in a saucer-shaped depression in the substratum (in artificial culture these form leathery or gelatinous, fertile hyphal mats)	MELANCONIALES.
Conidiophores free, arising irregularly from the mycelium	HYPHOMYCETALES.

The three orders correspond roughly with the three main divisions of Ascomycetes, Sphæropsidales with Pyrenomycetes, Melanconiales with Discomycetes and Hyphomycetales with Plectomycetes. Species belonging in the first two orders are seldom encountered in industrial work. They include many plant parasites and a number of saprophytes found on decaying plant material. Occasionally they appear in the laboratory in mixed cultures from infected material, but only as isolated colonies having no practical significance. Fig. 32 shows a typical member of the Sphæropsidales, with dark coloured pycnidia which, on crushing, exude an irregular mass of small hyaline spores (cf. Fig. 31). The Melanconiales, in their natural habitat, form a conidial layer, consisting of a crowded mass of short conidiophores, arising from a web of hyphæ occurring in a shallow depression in the surface of the host. The fructification somewhat resembles a pycnidium which has

been slit and opened out flat. When grown on artificial media such species produce cushion-shaped masses of short conidiophores, usually dark coloured, which fall to pieces when handled.

The great majority of the common moulds belong to the Hyphomycetales. Saccardo divides these into four families as follows:

1. Conidiophores detached, not compacted.
 - (a) Hyphæ colourless or in pale or bright colours. MUCEDINACEÆ.
 - (b) Mycelium, spores or both dark brown to black DEMATIACEÆ.
2. Conidiophores compacted in fascicles.
 - (a) Conidiophores short, forming cushion-shaped aggregates (sporodochia), often waxy to gelatinous TUBERCULARIACEÆ.
 - (b) Conidiophores long, forming coremia STILBACEÆ.

Each family is sub-divided into a number of sections according to the various types of spores.

Spores pale or brightly coloured

- | | |
|---|--------------------------|
| Spores 1-celled, globose, ovate or fusiform. | <i>Hyalosporæ.</i> |
| Spores 2-celled | <i>Hyalodidymæ.</i> |
| Spores with 2 or more cross septa | <i>Hyalophragmiæ.</i> |
| Spores muriform (i.e. with both longitudinal and cross septa) | <i>Hyalodictyosporæ.</i> |
| Spores spirally curved, septate | <i>Hyalohelicosporæ.</i> |
| Spores forked or star-shaped | <i>Hyalostaurosporæ.</i> |

Spores dark coloured

The classification is like that of the first group, the various names of sections having the prefix phæo- instead of hyalo-.

Each of the spore sections is then split into two groups.

1. Conidiophores short or obsolete, hardly distinguishable from the mycelium *Micronemææ.*
2. Conidiophores elongate and distinct *Macronemææ.*

Within these groups the various sub-families and genera are classified according to the arrangement of spores on the conidiophores, whether solitary or in heads, produced directly on the conidiophores or on specialized branches, arranged in clusters or in chains and so on.

In addition to the multitude of species falling within the

above scheme there are a number of fungi which are, so far as is known, permanently sterile. They produce mycelium and, in many cases, sclerotia, but no reproductive organs or conidia. These have no place in any of the families or groups founded on spore characteristics and are usually classed apart as *Mycelia sterilia*. Fig. 33 is a photograph of such a species which has been fairly frequently met with. It forms a web of greyish hyphæ, rapidly covering the surface of the medium, with numerous sclerotia, black, irregularly placed and of various shapes. Purely mycelial forms occur fairly frequently in miscellaneous cultures, but it should not be hastily assumed that these belong with the *Mycelia sterilia*. Many of them are parasitic fungi, Ascomycetes and Basidiomycetes, which do not form spores on the usual artificial media. In addition, many of the *Fusaria* form nothing but mycelium when first isolated and can be recognized only from experience or after cultivation under conditions which will induce normal production of spores.

Saccardo's system is, of course, a key pure and simple and not in any sense an attempt at a rational classification. The artificial nature of the criteria separates, in many cases, genera which are obviously closely related, but this of itself is not a serious matter, since the sole purpose of the key is to facilitate identifications. A graver fault is that some of the characteristics used as criteria are not so clear cut, in the majority of cases, as such a scheme demands, with the result that it is often necessary to search in more than one section of the key before a fungus can be identified. Perhaps the most serious weakness arises from the fact that numerous genera were, of necessity, known to Saccardo only from descriptions, many of which were totally inadequate for characterization. Some of these genera have been more completely defined by modern workers, but there are still a number of common moulds which are known to present-day mycologists merely from tradition handed on from one worker to another, and are extremely difficult to track down from keys founded on Saccardo's system.

Of the many attempts to devise a system of classification which shall be more logical than Saccardo's only one is of sufficient importance to be mentioned here. Vuillemin's system (1910, 1912) is based on differences in methods of spore

formation instead of on the characteristics of the spores themselves. It has been worked out most fully for a group of fungi which do not fit at all well into Saccardo's scheme, the pathogenic fungi, and is in general use by medical mycologists. The system has much to commend it and would probably be widely used if it could be extended to include the whole of the Fungi Imperfecti. Since the study of the pathogenic moulds is a very specialized branch of mycology, with its own extensive literature, Vuillemin's system will not be elaborated here.

Actinomycetes.

The place of these organisms in any scheme of classification is still in doubt, but if they are to be considered as fungi then their place is in the Fungi Imperfecti. The general consensus of present opinion seems to be that they form a connecting link between the bacteria and the true fungi. They resemble bacteria in staining reactions and in their inability to grow in presence of acid. In addition, some of the pathogenic species are anærobic and thus differ sharply from the true fungi, none of which can grow in complete absence of oxygen. On the other hand they form mycelium which shows true branching and in this respect are more closely allied to the fungi than to the bacteria.

A number of Actinomycetes are pathogenic to man and other animals, the study of these being usually regarded as the province of the bacteriologist. Other species are of common and widespread occurrence in soil and play an important part in maintaining its fertility, whilst a few are causes of plant diseases, usually known as "scab." In industrial work Actinomycetes commonly appear when plant products or soil contaminated materials are plated out. In such cases they may infect cultures of moulds sown from mixed plates and are often exceedingly difficult to eradicate.

Owing to their sensitiveness to acid they do not grow on wort agar and only a few species appear on Czapek agar containing KH_2PO_4 , which has a $p\text{H}$ of approximately 4.3. Many more of the common saprophytes, however, grow well on neutral Czapek agar, made up with K_2HPO_4 .

Colonies on agar media are usually slow growing and are mainly of two types. One kind is somewhat shiny and moist, often wrinkled, and in appearance closely resembling colonies

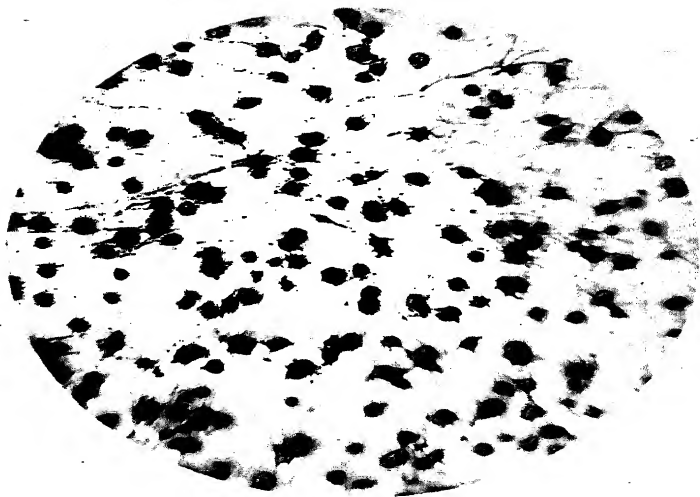


FIG. 33.—*Mycelium sterilium*—irregularly shaped sclerotia in hyphal web (Petri dish culture). $\times 20$.

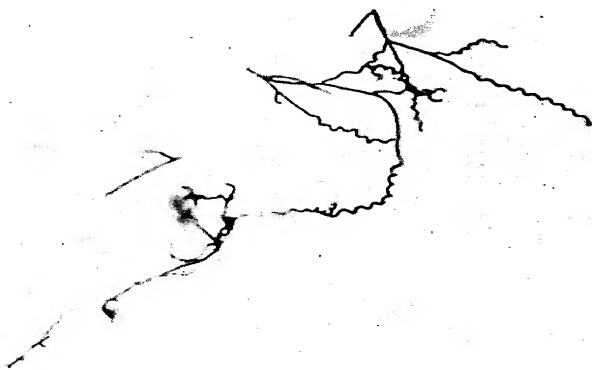


FIG. 34.—*Actinomyces* sp.—conidiophores with spiral chains of conidia (Petri dish culture). $\times 200$.

of certain *Torulæ*. Most of the parasitic forms but comparatively few of the soil organisms are of this type. The second and more common type of colony is tough and leathery, usually with well-defined margin, often becoming powdery on the surface as spores are formed. Colonies are variously coloured, white, grey, buff, yellow, greenish, red, brown or almost black. Some species form variously coloured soluble pigments which diffuse into the culture medium, but the extent to which this occurs depends a good deal on the type of substrate. Odour is usually pronounced and characteristic, not unlike that of moist loam but often more sour and unpleasant.

The mycelium is exceedingly fine, seldom more than 1μ in diameter, and much branched, with the terminal branches segmenting to form spores. In many of the common forms the spore-forming branches become spirally coiled before segmentation occurs (Fig. 34).

Three genera are recognized by most authorities, but all the species likely to be encountered in industrial work belong to the one genus *Actinomyces*.

Detailed accounts of the Actinomycetes are given by Lieske (1921), Ørskov (1923) and Waksman (1931).

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CHAPTER VI

FUNGI IMPERFECTI (*continued*)

Mucedinaceæ

The moulds of this family are characterized by colourless or pale or brightly coloured hyphæ and spores. In a very few cases, notably the *Aspergillus niger* group, the spores are dark coloured, but even here the vegetative and aerial hyphæ are hyaline, or at most yellowish, and the morphological similarity of these species to the rest of the genus is so obvious that there would be no point in excluding them on the sole ground of their having black spores.

The total number of generic names is very large, but it is probable that many of these will sooner or later be shown to be synonyms. Many genera have been founded on insufficient data, obtained from study of single specimens in their natural habitat. In most of such cases descriptions have been hopelessly inadequate and have not taken into account the whole life histories of the fungi. The result has been that some of the commoner moulds are known by tradition and are difficult to recognize from published descriptions, whilst many genera exist only as names and are totally unrecognizable at the present day.

The simple key given here takes into account only the more common forms, such as are fairly often found in industrial work. For identifications of fungi which are not included, the reader should consult one of the works of reference cited in Chapter XIV.

1. Spores one-celled, globose, ovoid,
elliptical or elongate. 2
Spores two-celled (in small clusters
on ends of erect conidiophores). *Trichothecium*.
2. Reproduction by fragmentation of
mycelium. No distinctive repro-
ductive organs. *Oidium* and *Oospora*.

Spores distinct from mycelium	3
3. Reproduction by ovate spores, increasing by budding and forming branched chains (also by fragmentation of mycelium in old cultures)	<i>Monilia.</i>
Spores always distinct from mycelium	4
4. Spores borne on all parts of mycelium, usually more or less pear-shaped	<i>Sporotrichum.</i>
Spores borne on definite conidiophores	5
5. Spores in loose clusters on irregularly branched conidiophores	<i>Botrytis.</i>
Spores in more or less globular clusters, borne directly on ends of conidiophores	6
Spores borne in chains, formed by abscission from special spore-bearing organs (sterigmata)	8
6. Conidiophores unbranched, mostly very short	<i>Cephalosporium.</i>
Conidiophores branched	7
7. Branching of conidiophores irregular, not verticillate	<i>Trichoderma.</i>
Conidiophores verticillately branched	<i>Verticillium.</i>
8. Conidiophores arising from specialized foot-cells, usually non-septate, terminating in a swelling which bears the sterigmata	<i>Aspergillus.</i>
Conidiophores not arising from foot-cells, septate	9
9. Conidiophores branched irregularly	10
Conidiophores with verticillate branching, broom like	11
10. Spores large, with thickened base and pore	<i>Scopulariopsis.</i>
Spores oval, without ring and pore, sterigmata ending in long beaks	<i>Pæcilomyces.</i>
11. Spores chains enveloped in mucilage	<i>Gliocladium.</i>
Chains free, mucilage absent	<i>Penicillium.</i>

Oidium and Oospora. There is some difference of opinion as to the use of the two names. Many authorities reserve the name *Oidium* for parasitic forms, mostly imperfect stages of Erysiphaceæ and known as powdery mildew, and the name *Oospora* for saprophytes. Others regard species in which the mycelial fragments are more or less rectangular as *Oidium* and species with rounded cells as *Oospora*. It is probably best to follow the former practice.

Oospora lactis (Fresenius) is well known in the dairy industry and is found on all types of milk products. It grows well on wort agar, producing a thin, spreading, somewhat slimy, creamy white colony. Very young cultures show fairly long mycelial strands, whilst older cultures consist entirely of short rectangular fragments, which take the place of spores (Fig. 35).

O. crustacea Bulliard is commonly found on cheese rind, where it forms an orange-coloured, powdery growth. On culture media it grows best at about 18° C., spreading slowly at first, then more rapidly, the colour being bright orange to scarlet. At higher temperatures many strains readily form saltants, dark brown or white, which do not revert to the orange form. The cells of the young mycelium become rounded before falling apart, and look like long chains of ovate conidia (Fig. 36).

Morilia. One species, *M. sitophila* (Montagne) Saccardo, is of common occurrence and can be a great nuisance in a culture room since it spreads with extreme rapidity on most media. It forms loosely floccose masses of a pale pink to salmon-pink colour. Ovate conidia are borne at the ends of the aerial hyphæ (Fig. 37). These increase by budding and eventually form enormous, irregular masses (Fig. 38). Later the mycelium breaks up at the septa, though not usually so freely or completely as *Oospora*. Shear and Dodge (1927) have shown that *M. sitophila* is the imperfect form of an Ascomycete, *Neurospora sitophila*, but, since the latter is heterothallic, it is seldom that ascospores are found.

Sporotrichum. Several species are known, most of them saprophytes, but at least one species is parasitic on man, causing a skin disease known as sporotrichosis. The saprophytic forms grow as cream-coloured colonies, at first wet and yeast-like, later becoming dry and more or less powdery.



FIG. 35.—*Oospora lactis*—fragmenting mycelium and oidia. $\times 250$.



FIG. 36.—*Oospora crustacea*—chains of oidia (from slide culture). $\times 250$.

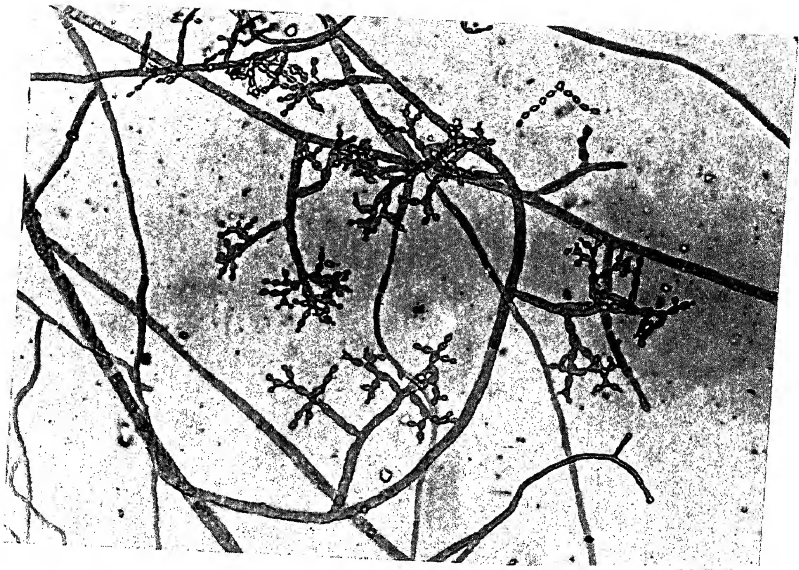


FIG. 37.—*Monilia sitophila*—young culture (on slide) showing budding of spores. $\times 200$.



FIG. 38.—*M. sitophila*—spore masses as seen in culture tube. $\times 100$.

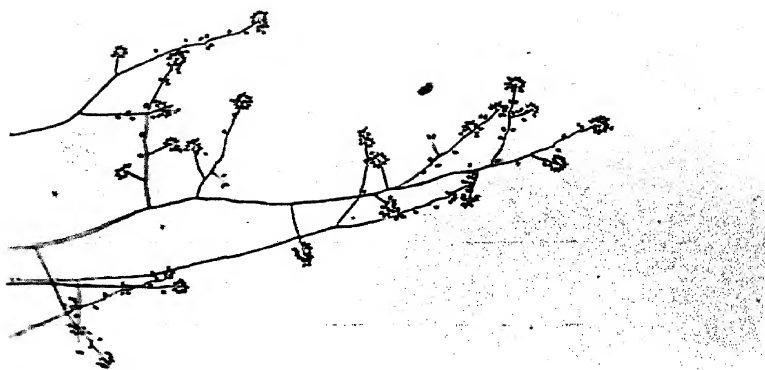


FIG. 39.—*Sporotrichum* sp.—conidia (slide culture). $\times 250$.



FIG. 40.—*Cephalosporium* sp.—fertile hyphæ with small spore-balls, as seen in living culture. $\times 100$.

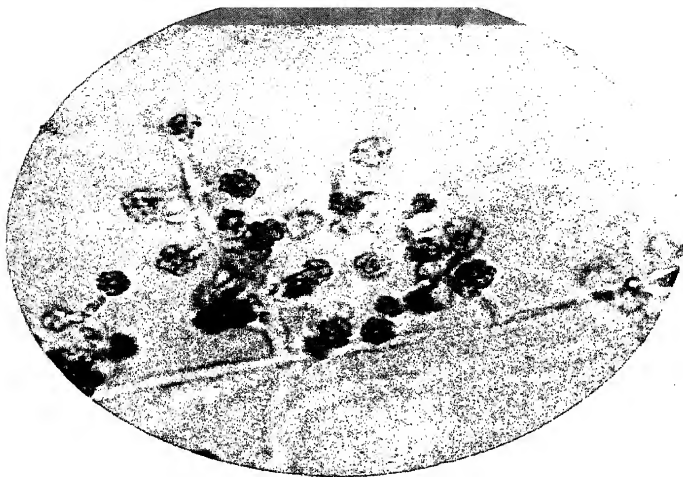


FIG. 41.—*Trichoderma lignorum*—conidiophore with balls of spores (slide culture.) $\times 500$.



FIG. 42.—*Verticillium glaucum*—young conidiophore as seen in Petri dish. $\times 200$.



FIG. 43.—*Botrytis cinerea*—typical conidiophores. $\times 100$.

Spores, mostly small and pear shaped, are produced singly or in small clusters from all parts of the mycelium (Fig. 39).

Cephalosporium. In this genus the conidiophores are short branches from trailing hyphæ, bearing at their tips approximately spherical masses of spores, which are usually held together by a sticky fluid (Fig. 40). The spores are produced in great numbers and the balls fall to pieces when touched. A number of species have been described, but they are mostly ill-defined and difficult to identify. The forms usually encountered spread very rapidly on laboratory media and when occurring, as they often do, as contaminants of other cultures, are not easy to eradicate.

Trichoderma. The spores are produced as in *Cephalosporium*, but the conidiophores are irregularly branched. *T. lignorum* (Tode) Harz is of common occurrence on fallen timber and frequently appears in the laboratory on all kinds of infected material. It spreads rapidly, forming a somewhat thin mycelium with irregularly shaped patches of a bright verdigris green, this being the colour of the spores in mass. The fragile, spherical heads contain 10 to 20 spores, 2.5μ to 3μ in diameter (Fig. 41).

Verticillium. The spores are borne as in *Trichoderma*, but the conidiophores are longer and verticillately branched (Fig. 42). *V. glaucum* Bonorden forms rapidly spreading colonies of a dense cottony texture, gradually turning glaucous green in patches as the spores ripen. *V. cinnabarinum* (Corda), commonly known as *Acrostalagmus cinnabarinus*, forms thin, velvety colonies of a brick-red colour, with elliptical spores, about 3μ by 1.5μ .

Botrytis. Only one species, *B. cinerea* Persoon, is of frequent occurrence. It is a common saprophyte and also a serious parasite of many kinds of plants. In culture it grows as floccose colonies, pale, dirty brown in colour, with fairly long, stiff conidiophores, irregularly branched at the end and bearing clusters of ovate conidia, the whole fructification resembling a bunch of grapes (Fig. 43). Typical sclerotia begin to develop after a few days as small knots of mycelium, which rapidly increase in size, turn dirty green, then black and finally may attain several millimetres in diameter.

***Trichothecium*.** There is one common species, *T. roseum* Link (Syn. *Cephalothecium roseum* Corda). Colonies are somewhat thin, floccose, wide spreading, white at first, then slowly pale pink. The erect conidiophores bear terminal clusters of spores directly attached to the tip (Fig. 44). The spores are 2-celled, roughly oval with a nipple-like projection at the point of attachment, $18-20\mu \times 8-10\mu$ (Fig. 45).

Of the remaining genera in this family, *Aspergillus* and *Penicillium* are of such outstanding importance that they are considered separately in Chapters VII and VIII, *Gliocladium*, *Scopulariopsis* and *Pæcilomyces* being included with *Penicillium*.

Dematiaceæ

The moulds of this family are distinguished from the Mucedinaceæ by their dark colour, the mycelium, spores or both being dirty brown or black. Many of the common species are readily recognized as belonging here by their uniformly sooty colour, but a few species have pale-coloured vegetative hyphæ and show the typical colour only in the reproductive organs. A large number of the Dematiaceæ which are dark coloured when first isolated from natural substrata tend to produce a certain amount of pale-coloured, cottony aerial mycelium when grown on artificial media. With continued cultivation this tendency increases, production of spores becomes more and more scanty, and, unless such fungi are subcultured on to vegetable or other special media, they degenerate completely, losing their dematiaceous character and becoming quite unrecognizable.

KEY TO IMPORTANT GENERA

Spores one-celled

Spores increasing by budding, forming branched chains, becoming 2-celled in old cultures *Cladosporium*.

Spores borne on sterigmata, the latter in whorls *Stachybotrys*.

Spores elongate, with two or more cross septa, borne singly or apparently in clusters on tips of conidiophores.

Spores smooth *Helminthosporium*.

Spores warted *Heterosporium*.

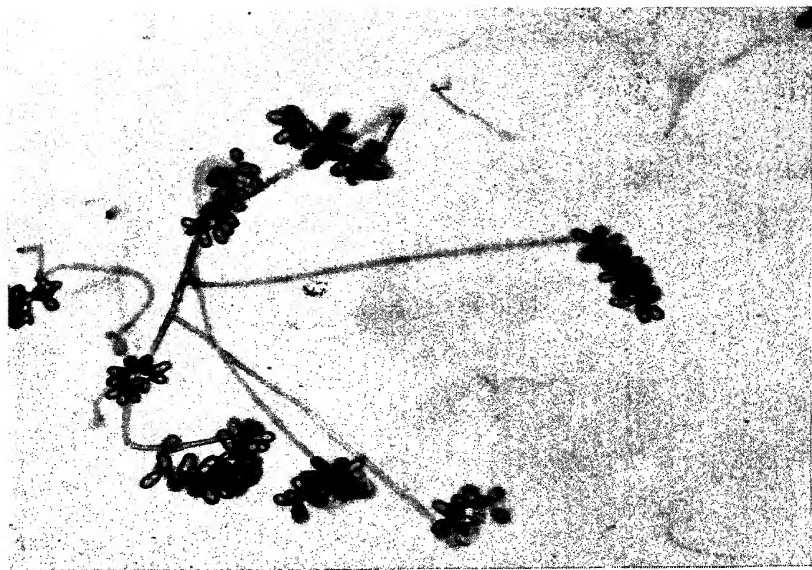


FIG. 44.—*Trichothecium roseum*—conidiophore as seen in dish culture. $\times 200$.

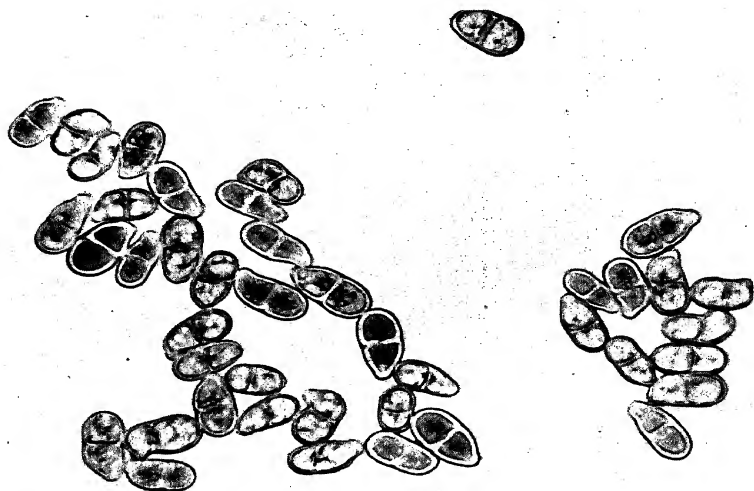


FIG. 45.—*T. roseum*—spores. $\times 500$.

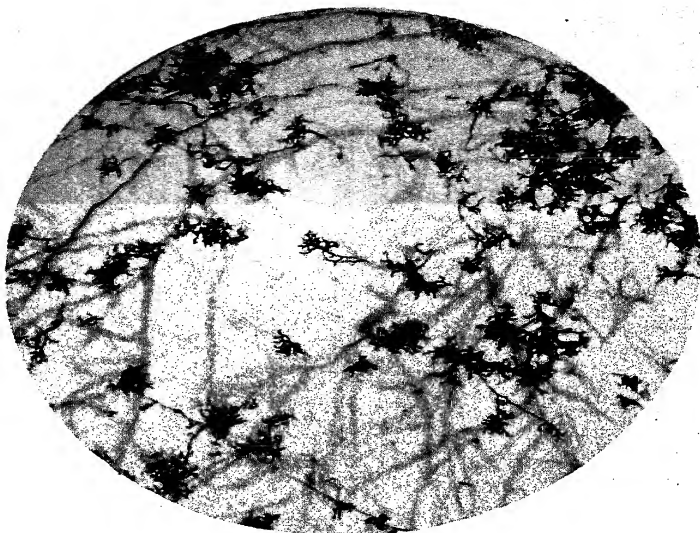


FIG. 46.—*Cladosporium herbarum*—spore heads as seen in dish culture. $\times 50$.

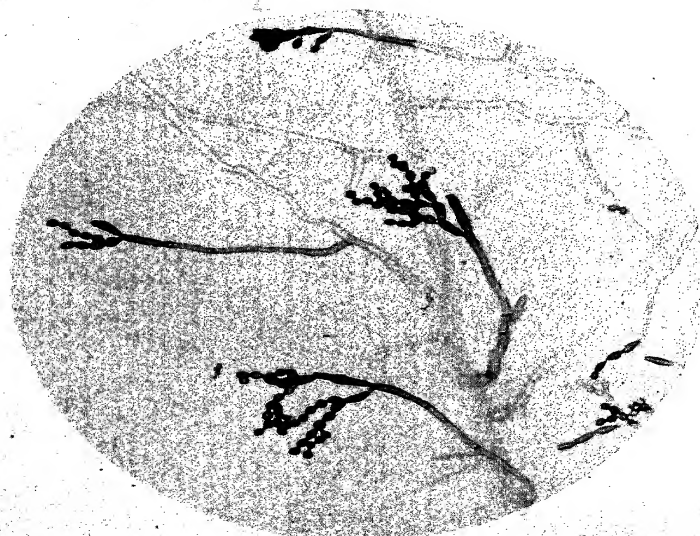


FIG. 47.—*C. herbarum*—young conidiophores showing budding of spores (slide culture). $\times 250$.

Spores muriform, i.e. with both cross and longitudinal septa.

Spores in short chains, somewhat elongated,
always pointed or beaked *Alternaria*.

Spores in clusters, rounded, not beaked . . . *Stemphylium*.

***Cladosporium*.** A large number of specific names have been bestowed in this genus, but it is certain that the majority are to be regarded as synonyms. A few of the parasitic forms are fairly readily distinguished but, until such time as someone sorts out the genus according to modern ideas, it is probably best to lump together all the common, green, saprophytic forms as *C. herbarum*. *C. herbarum* Link is a common organism of very widespread occurrence, being found on textiles, rubber, leather, numerous foodstuffs and all kinds of decaying materials of vegetable origin. It grows over a wide range of temperature, and has been reported as infecting meat stored at -6°C . On culture media the usual type of colony is somewhat restricted in growth, thick velvety, in colour varying from deep rich green to dark grey green, with reverse a curious opalescent blue black or greenish black. When examined dry under the microscope, the spores are seen to occur in large, almost tree-like clusters (Fig. 46). When mounted, the structure breaks up completely, the spores becoming detached and the hyphæ breaking into short rod-like fragments. All parts of the fungus are dark brownish green to dark brown. Examination of very young cultures will show that the ovate spores increase by budding (Fig. 47) in a manner reminiscent of the yeasts, eventually forming much-branched chains. The young spores are mostly non-septate, the older ones frequently 2-celled. Originally the name *Cladosporium* was restricted to strains with septate spores, *Hormodendron* being the name given to forms with one-celled spores, but it is now known that both types of spore occur regularly in a single culture and, therefore, the name *Hormodendron* becomes a synonym.

***Stachybotrys*.** The vegetative hyphæ and conidiophores are hyaline or nearly so. The short conidiophores bear at their tips whorls of thick sterigmata, hyaline or partially brown, each bearing a spore or a short chain of spores. The spores are elliptical to sub-globose, often slightly curved, brown or black

and usually warted. *S. atra* Corda, with spores 8–9 μ long, is fairly common on paper and other cellulosic materials (Fig. 48).

***Helminthosporium*.** All of the many species of this genus are best known as parasites of cereals and grasses. Many of them, however, grow well as saprophytes and are not uncommon on vegetable materials. In culture some species grow normally, with dark-coloured hyphæ and typical spores. Others appear to be quite sterile and tend to produce considerable amounts of hyaline or pale-coloured mycelium. It has been shown by Raistrick and co-workers (see Chapter XIII) that certain species of the latter group, when grown on Czapek-Dox solution, synthesize red or purple pigments which are hydroxyanthraquinones and are closely related to the parent substances of some of the most valuable of the fast dyes. In all the published keys to the Dematiaceæ *Helminthosporium* is given as producing spores singly at the ends of the conidiophores, whereas examination of a sporing culture will usually show clusters of spores resembling bunches of bananas. Actually the short, stiff conidiophore bears first a single spore slightly to one side of the tip. The tip then elongates somewhat and bears a second spore in like manner and this process may continue until a dozen or more spores have been formed (Fig. 49). Sometimes the intervals are fairly long, and the method of their formation is readily observed, but often the spores are closely packed and appear to be in true clusters. The spores are fairly long, roughly cylindrical, often somewhat attenuated at the ends, smooth, dark coloured, with many cross septa. None of the species are of appreciable importance in industrial work and those wishing to identify them should consult the papers of Drechsler (1923) and Nisikado (1929).

Heterosporium is similar to *Helminthosporium*, but the spores are mostly somewhat shorter and are distinctly rough. All the species are parasitic and the genus is included here merely because occasional cultures appear when mouldy materials are plated out in the laboratory.

***Alternaria*.** Some species are of common occurrence on numerous kinds of organic material under damp conditions, whilst others are parasitic on cultivated plants. In culture on rich media most forms spread rapidly, with densely floccose, dirty green mycelium. On poor media growth is less floccose



FIG. 48.—*Stachybotrys atra*—conidiophores, showing sterigmata and dense masses of dark spores. $\times 500$.



FIG. 49.—*Helminthosporium monoceras*—young conidiophores, showing "successive" formation of spores.

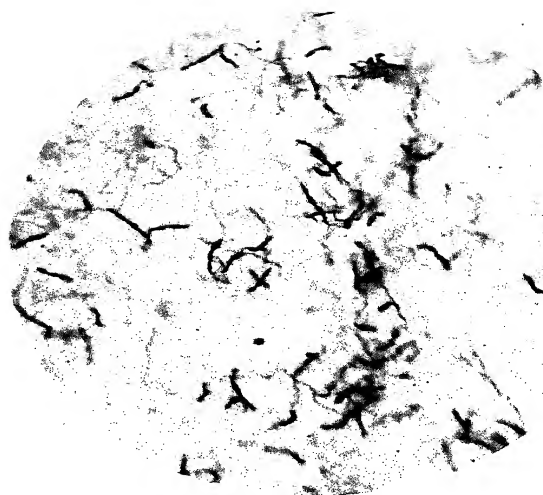


FIG. 50.—*Alternaria brassicae*—spore chains as seen in dish culture. $\times 42$.

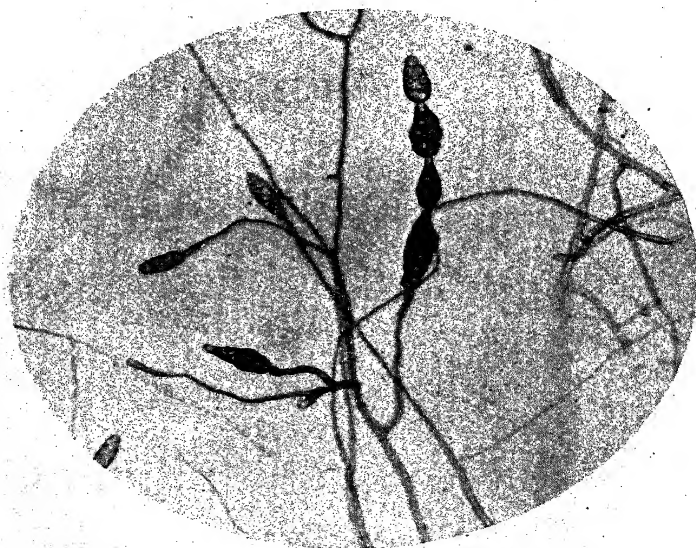


FIG. 51.—*A. brassicae*—typical short chain of spores (slide culture). $\times 250$.



FIG. 52.—*Alternaria tenuis*—spores from culture on poor medium. $\times 250$.

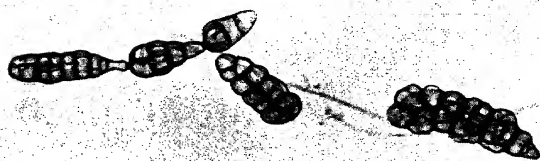


FIG. 53.—*A. tenuis*—spores from culture on rich medium. $\times 250$.



FIG. 54.—*Stemphylium* sp.—as seen in dish culture. $\times 65$.

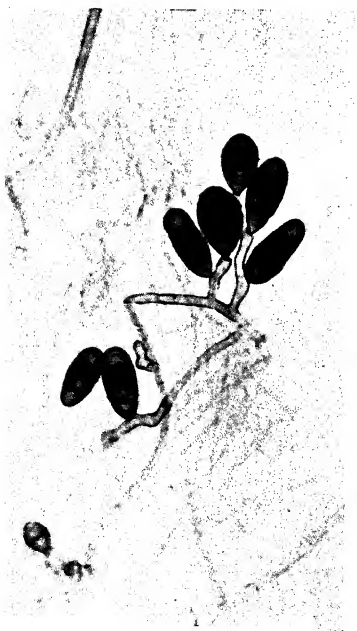


FIG. 55.—*Stemphylium* sp.—short conidiophore with cluster of spores. $\times 500$.



FIG. 56.—*Stemphylium* sp.—spores showing a very usual type of septation. $\times 250$.

and darker in colour. The mycelium is very richly septate and often forms chains of short, swollen cells not unlike oidiospores. The conidia vary in shape from roughly ovate to inverted club-shaped, with a more or less pronounced beak at the apex. They are produced in short chains, often branched, are greenish brown to dark brown in colour and have both cross and longitudinal septa, the degree of septation increasing with age (Figs. 50, 51). On rich culture media spore production is usually sparse, but the individual spores are large and elaborately septate (Figs. 52, 53). A paper by Elliot (1917) has simplified the taxonomy of what was previously a difficult and confusing genus and should be read by those who are interested in *Alternaria* and the related genera, *Macrosporium* and *Stemphylium*. For a few species of *Alternaria* the perfect stage is known, in every case belonging in *Pleospora*.

***Stemphylium*.** Species of *Stemphylium* are common saprophytes, frequently being found in association with *Cladosporium* and *Alternaria*. The conidiophores occur as short branches from trailing hyphæ, bearing the spores in small clusters (Figs. 54, 55). The spores are rounded, not beaked, and usually very dark in colour. They mostly have comparatively few septa and are often sarceniform (Fig. 56).

Stilbaceæ

The family includes fungi in which the conidiophores are predominantly aggregated into coremia. The latter may consist of stalk and head, the stalk being a bundle of conidiophores and the head a bushy mass of fruiting structures, or may consist of a compact rope of hyphæ bearing short fertile branches along the whole length. Both pale- and dark-coloured species are included. The family is a small one and, even then, includes some genera which have been described from somewhat abnormal forms of such well-known fungi as *Penicillium*.

***Coremium*.** The type species, *C. glaucum* Link, is most probably identical with *Penicillium expansum*, which, under special conditions, produces large, well-formed coremia. *C. sylvaticum* Wehmer is similarly a synonym of *P. claviforme* Bainier.

***Isaria*.** Species of this genus are best known as parasites of insects, but a few forms are able to grow on ordinary culture

media, where they form long white coremia, approximately cylindrical and fertile along the whole length (Figs. 57, 58).

***Stysanus*.** One species, *S. stemonitis* Persoon, is encountered sufficiently often to be mentioned here. Colonies are greyish black in colour and consist chiefly of densely crowded coremia. The latter have slender stalks, and cylindrical to club-shaped heads, with ovate spores about 7μ in long axis. (Fig. 59.)

Tuberculariaceæ

The typical fructification is a cushion-shaped aggregate of short conidiophores (the sporodochium), in effect a stalkless coremium, often arising from a leathery or waxy mycelial web known as a stroma. Only one genus is of any importance outside the province of plant pathology.

***Fusarium*.** Taxonomically this is the most difficult of all the genera of mould fungi. Not only is the number of species very large but most species are difficult to maintain in a state of stability. Wollenweber, who has done more than any other worker to clear up the *Fusarium* problem, has stated that *Fusaria* pass through certain well-defined stages when kept in cultivation. Under favourable conditions they normally pass from a mycelial to a freely sporing stage, and thence to a degenerate state with spores abnormal and sparingly produced. Another view is that the whole question of the changes which take place can be explained by the readiness with which many species produce saltants, the relative vigour of these determining the type of growth propagated if no particular care is taken to separate the forms when sub-culturing. The latter opinion is supported by the fact, observed by many workers, that transfer of spores usually will produce sporing cultures, whilst transfer of mycelium will tend to retard spore production and give completely sterile colonies. For culture of *Fusaria* so as to retain as far as possible desired characteristics Wollenweber *et al.* (1925) recommend various media made from vegetable materials. Brown (1925), however, prefers a synthetic medium containing glucose, 2 g.; asparagine, 2 g.; K_3PO_4 , 1.25 g.; $MgSO_4$, 0.75 g.; agar, 15 g.; water to 1 litre. The only disadvantage of this is that, owing to its containing only a very small percentage of total solids, it dries out quickly and shrinks to a mere streak on the glass of the culture tube if

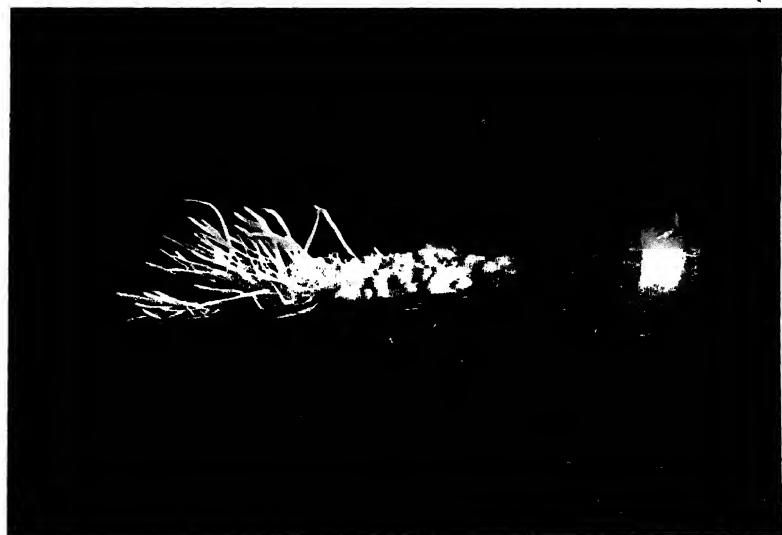


FIG. 57.—*Isaria* sp.—culture on agar slope.
Natural size.



FIG. 58.—*Isaria* sp.—branched coremium. $\times 12.5$.



FIG. 59.—*Stysanus stemonitis*—typical
coremium on agar slope. $\times 50$.

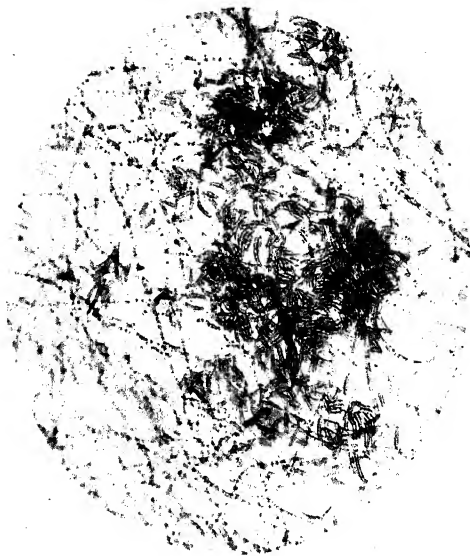


FIG. 60.—*Fusarium* sp.—clusters of conidia as seen in dish culture. $\times 100$.



FIG. 61.—*Fusarium* sp.—cluster of conidia, a few containing chlamydospores. $\times 500$.



FIG. 62.—*Fusarium* sp.—typical conidia. $\times 1000$.



FIG. 63.—*Fusarium* sp.—chlamydospores in mycelium. $\times 250$.

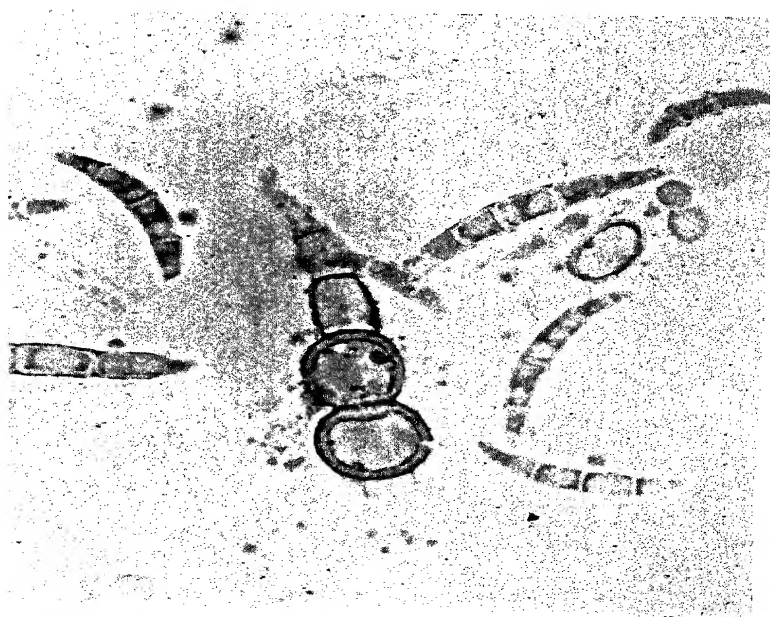


FIG. 64.—*Fusarium* sp.—chlamydospores conidium. $\times 1000$.

cultures are kept for more than a few weeks. The best of the common media, for maintenance of stock cultures, is ordinary potato agar.

Many *Fusaria*, when first isolated, produce mycelium alone and it is often a matter of some difficulty to induce production of normal conidia for purposes of identification. Some will spore when the cultures become old and dry, others respond to a starvation diet such as tap-water agar, whilst some seem to need a particular medium which can only be found by trial. If such mycelial cultures are kept on media containing a fairly high percentage of sugar, such as wort or Czapek agar, they tend, after several sub-culturings, to degenerate to a slimy stage, consisting of colourless, gelatinous masses of anastomosing hyphæ, and are then almost impossible to restore to the normal form.

In all systems of classification *Fusarium* is included with the Tuberculariaceæ, but many species produce, instead of sporodochia, smooth, gelatinous layers of spores (pionnotes) and are thus more nearly allied to the Melanconiales, and still others form neither sporodochia nor pionnotes, but produce conidia in scattered clusters on all parts of the aerial mycelium. The one feature common to the different types is the spore. The typical spores, or macro-conidia of *Fusarium* are sickle-shaped, with pointed ends, usually with several cross septa, colourless or pale coloured, never dark or dematiaceous, and are quite unlike those of any other mould fungi (Figs. 60, 61, 62). The exact shape, size and number of septa in the macro-conidia are of importance in determining species. In addition to the macro-conidia many species produce micro-conidia which are small, ovate, elongate, pyriform or comma-shaped, usually non-septate, more rarely 1-3 septate. A number of species form chlamydospores, which may be terminal, intercalary or both, formed in the mycelium or in the macro-spores (Figs. 63, 64). Sclerotia or sclerotial masses are not uncommon and are often brightly coloured. As in other genera, all such features have diagnostic importance.

The perfect stages of a fairly large number of *Fusaria* are now known. They are all Ascomycetes, and belong to the genera *Hypomyces*, *Gibberella*, *Nectria* and *Calonectria*.

Most of the taxonomic work on *Fusarium* has been done in

connection with plant diseases and there is an extensive literature of such. The identification of species occurring as parasites is now, since the publication of the monograph by Wollenweber and Reinking (1935), comparatively easy, since it is known that the number of possible species found on any particular plant is small, and the pathological symptoms are fairly definite for each species. In industrial work, however, it is quite otherwise. Most of the *Fusaria* grow readily as saprophytes and are found in almost every situation where moulds of any kind can grow, but the difficulty of identification is still very great and, until recently, was a task for the specialist alone. In the literature of applied mycology there are numerous references to "*Fusarium* sp." but very few, if any, references to the occurrence of particular species on industrial products. Until such information is available it would be useless to distinguish any of the species by description here.

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CHAPTER VII

ASPERGILLUS

Species of this genus form a very large proportion of all the moulds encountered in industrial work. They are to be found almost everywhere on every conceivable type of substratum, and a good working knowledge of the genus is essential to anyone undertaking serious work on problems of mouldy deterioration. The activities of the *Aspergilli*, however, are not entirely destructive in nature, for several species have had their fermentative powers harnessed for commercial purposes. In the East, strains of *A. oryzae* have long been used for the saccharification of rice starch in the production of saké and similar potable liquors. Strains belonging in the same group are used in the manufacture of soy sauce and of the enzymic mixtures sold under the names "Takadiastase" and "Polyzime." The black *Aspergilli*, long known to be capable of producing considerable amounts of oxalic acid from sugar, are now used for the successful commercial production of citric acid, following Currie's discovery that, by suitable adjustment of conditions, the formation of oxalic acid can be inhibited and the production of the more valuable citric acid encouraged.

The following is a generic diagnosis sufficiently broad to cover all the species of any importance.

Aspergillus. Mycelium hyaline or bright or pale coloured, or bearing surface concretions of colouring matter, never dematiaceous (black or smoky brown), septate, partly submerged and partly aerial; fertile branches (stalks) arising from and more or less perpendicular to specialized, thick-walled, enlarged mycelial cells (the foot-cells), mostly non-septate, smooth, roughened or pitted, frequently enlarging towards the apex and terminating in a swelling (the vesicle) which may be

variously globose, sub-globose, clavate, hemispherical, or a mere thickening of the stalk; specialized conidium-bearing cells (sterigmata) produced simultaneously, radiating from the whole or upper part of the vesicle, bearing conidia directly, or each primary sterigma bearing a cluster of secondary sterigmata, which latter then bear the conidia; conidia successively cut off by cross walls from the continuously elongating tips of the sterigmata, thus forming unbranched chains, variously shaped and coloured; ripe spore heads of various shapes—globose, radiate, clavate or columnar—varying much in size in different species; perithecia produced by only a few species, thin-walled, breaking up to liberate the ascospores; sclerotia known in several species but not of diagnostic importance.

Some of the smaller and more delicate species bear a strong resemblance to certain of the monoverticillate *Penicillia*, but there are two criteria by which such may be separated. Thom and Church regard the presence of foot-cells as the important distinguishing feature and, in spite of the fact that Smith (1933) has described two species of *Penicillium* with true foot-cells, the criterion is quite valid for the borderline species of the two genera. Fig. 65 shows a foot-cell with the stalk and head arising from it, and this may be compared with several photographs of *Penicillia* in the next chapter. The French school of mycologists consider the method of production of the sterigmata, simultaneous in *Aspergillus* (see Fig. 66), successive in *Penicillium*, to be of primary generic significance. In the opinion of the author, both distinctions are equally to be trusted, but it is sometimes very difficult to demonstrate the presence of foot-cells in just those borderline species, owing to their compact habit and the persistence of tangled masses of mycelium in microscopic mounts. On the other hand, it is usually easy to find, especially near the edge of a growing colony, partially developed heads showing various stages in the growth of the sterigmata.

Sterigmatocystis

Various authorities in the past have considered *Aspergillus* to include only the species with one series of sterigmata, and have separated as a separate genus, *Sterigmatocystis*, all the species with two series. More recently Biourge (1933) has

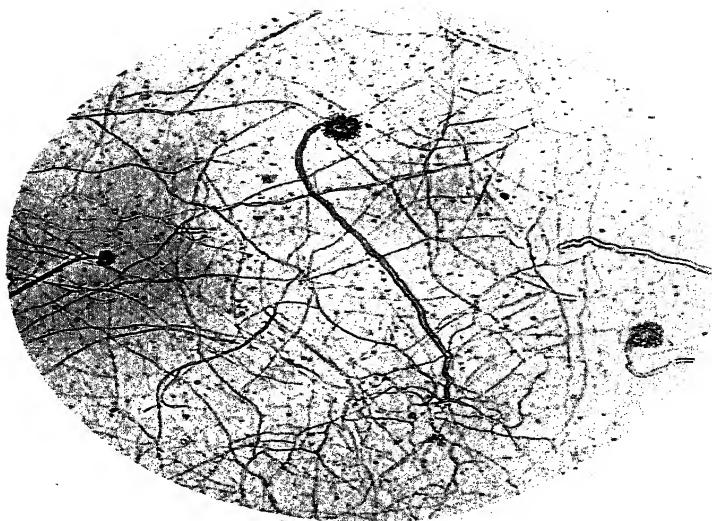


FIG. 65.—*Aspergillus candidus*—conidiophore with foot-cell (slide culture). $\times 100$.

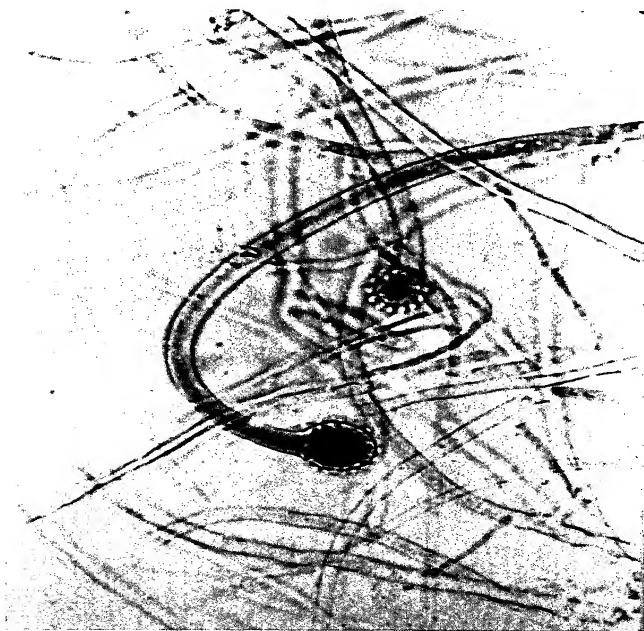


FIG. 66.—*A. candidus*—showing “simultaneous” production of sterigmata. $\times 500$.

upheld the separation. However, Wehmer and, later, Thom and Church (1926) have rejected *Sterigmatocystis* on the ground that, in several species, the number of series of sterigmata is not constant. In particular, strains of *A. tamarii* and *A. flavus* regularly produce two kinds of heads, with one and two series of sterigmata respectively, and it is not unusual to find simple and branched sterigmata in the same head.

Eurotium

The connection of the *A. glaucus* series with the ascomycetous genus *Eurotium* has been mentioned in the chapter on the Ascomycetes. From the systematic point of view there is much to be said for retaining the name of the perfect form but, in accordance with the consensus of modern opinion, the group of species is considered here as a section of *Aspergillus*. It should be noted that many references in the literature to this important group will be found under the generic name *Eurotium*.

Determination of Species. The taxonomy of the genus is, up to a point, not difficult owing, in large measure, to the excellent scheme of classification given in the monograph by Thom and Church. The genus, moreover, is probably unique amongst the common Hyphomycetales in the wide range of colony colour exhibited, the colours being comparatively stable and correlated with morphological and biochemical characteristics.

One of the most important factors in the clarification, by Thom and Church, of this great genus, is their conception of what may be termed the group species. Some species are fairly definite and of stable morphology. A single strain may be described in definite terms and other strains encountered will fit the description with considerable accuracy, justifying the conclusion that here we have a true species. In other cases, as in the series represented by *A. versicolor*, *A. candidus*, *A. flavus* and others, different strains, whilst clearly related, differ somewhat widely in minor details. Anyone who collects only a few strains belonging in such a series may observe differences which seem to justify the creation of several species but, when scores or hundreds of strains are examined, the sharp lines of demarcation disappear and, instead of a few definite species, there is a perfectly gradated series such that no one

could describe any particular strain in terms which permit of its certain identification by another who had not the same series of strains before him. Delimitation of species in such a group is also complicated by the fact that a single strain may cover a considerable range in the series in response to variations in cultural conditions, or even in successive cultures grown under the same conditions. The specialist who is interested in one particular group will collect a number of strains and will know them sufficiently well to be able to separate them on grounds dictated by his own requirements. For most purposes, however, it is sufficient to be able to identify any particular culture as a member of one of the group species. After all, this so-called "lumping" of strains is only the equivalent of the recognition of variation amongst individuals belonging in any one species of flowering plant or animal.

KEY TO THE PRINCIPAL SPECIES OF ASPERGILLUS

The following key is based on the classification of Thom and Church and is, for brevity, not strictly dichotomous.

- | | |
|---|----|
| 1. Stalks smooth | 2 |
| Stalks pitted or roughened | 11 |
| 2. Vesicles elongated club-shaped <i>A. clavatus</i> . | |
| Vesicles otherwise | 3 |
| 3. Spore heads columnar | 4 |
| Heads radiate or globose | 7 |
| 4. Colonies green | 5 |
| Colonies sand brown, stalks colourless <i>A. terreus</i> . | |
| Colonies silvery white to pale buff, stalks yellowish <i>A. flavipes</i> . | |
| 5. Sterigmata in one series | 6 |
| Sterigmata in two series <i>A. nidulans</i> . | |
| 6. Conidia ovate, echinulate, mostly more than 4μ long <i>A. penicilloides</i> group. | |
| Conidia globose, mostly less than 4μ <i>A. fumigatus</i> . | |
| 7. Conidial areas some shade of green | 8 |
| Colonies never green | 10 |

8. Sterigmata in one series,
conidia ovate, echinulate ;
yellow perithecia present . . . *A. glaucus* series.
Sterigmata in two series
9. Colonies blue-green . . . *A. Sydowi*.
Colonies green or yellowish
green to buff *A. versicolor* group.
10. Spore heads buff to olive brown,
stalks coloured *A. ustus*.
Heads white to cream . . . *A. candidus*.
Heads dark brown to black . *A. niger* group.
Heads coffee-coloured, my-
celium forming dense floc-
cose masses *A. Wentii*.
11. Heads yellow, ochre or brown. 12
Heads green, yellowish green
or greenish buff. . . . *A. flavus-oryzæ* series.
12. Heads bright yellow . . . *A. sulphureus*.
Heads ochraceous. . . . *A. ochraceus*.
Heads greenish brown, becom-
ing deep brown, conidia
roughened by bars of colour-
ing matter *A. tamarii*.

The following descriptions are believed to be sufficient for recognition of the species but, in most cases, are not complete diagnoses.

The A. glaucus Series. Species included under this name are amongst the most commonly occurring and most destructive of all moulds. They are usually readily recognized by the presence of both bluish green heads and bright yellow perithecia.

Group diagnosis : Conidial areas mostly bright bluish green in young colonies, becoming dull green to brownish green, with the colour often partially masked by red or brown pigments encrusting the mycelium ; medium frequently coloured red, brown or purple ; stalks usually septate, smooth, thin-walled, often collapsing in mounts ; vesicles varying from mere thickenings of the stalk (Fig. 67) to more or less globose (Fig. 68) ; sterigmata in one series, covering the whole or only part of the vesicle, usually broad, often proliferating to form small short stalks bearing secondary heads (Fig. 69) ; conidia echinulate, rarely smooth, mostly greater than 5μ in long axis

but varying much in size and attaining, particularly at low temperatures, 15μ or even 20μ , usually ovate or pear-shaped and showing a distinct connective; heads loosely radiate, having a ragged appearance when old; perithecia (Fig. 70) yellow to orange, globose or nearly so, varying from about 80μ to 250μ in diameter, containing 8-spored asci in irregular arrangement; asci usually breaking up as spores mature; ascospores hyaline, biconvex, usually with a furrow marking the line along which the wall splits in germination, with the sides of the furrow rounded or raised into crests or frills.

The group is a difficult one for the taxonomist. There are a few fairly well-marked species and also a number of strains which are difficult to separate. Mangin (1909) divides the group on the basis of size and markings of ascospores and, although the number of strains studied (23) was not sufficient for a really adequate treatment, his scheme, with the addition of *A. ruber*, is well adapted for a preliminary sorting out.

Ascospores with well-marked furrow and mostly with crests
or frills, more than 7.5μ in long axis

A. herbariorum series major (Mangin).

Ascospores with furrow and crests or frills, 6.6 to 7.5μ in
long axis

A. herbariorum series minor (Mangin).

Ascospores with furrow and well-marked crests, 4.7μ by 3.3μ

A. Chevalieri (Mangin).

Ascospores with shallow furrow and rounded crests, 5.0 – 5.5μ
by 4.0 – 4.2μ (occasionally larger or smaller)

A. Amstelodami (Mangin).

Ascospores with smooth, rounded furrow and no crests,
 4.7μ by 3.7μ up to about 5.2μ by 4.0μ

A. repens (Corda) Saccardo.

Ascospores smooth, without any trace of furrow or crests,
 6.0μ by about 4μ

A. ruber (Spieckermann and Bremer).

All the species were originally described as belonging in *Eurotium*. Except in the cases of *A. repens* they have been transferred to *Aspergillus* by Thom and Church. Fig. 71, reproduced from Mangin's paper, illustrates, better than any description, his conception of the terms used. Bainier and Sartory have described a number of species which would fall within Mangin's two series of *A. herbariorum*; for details of

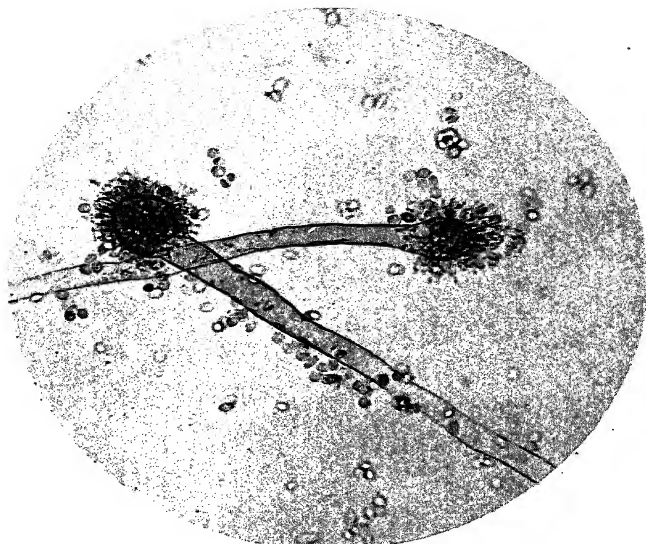


FIG. 67.—*A. glaucus*—heads with small vesicles. $\times 250$.



FIG. 68.—*A. glaucus*—heads with globose vesicles. $\times 500$.

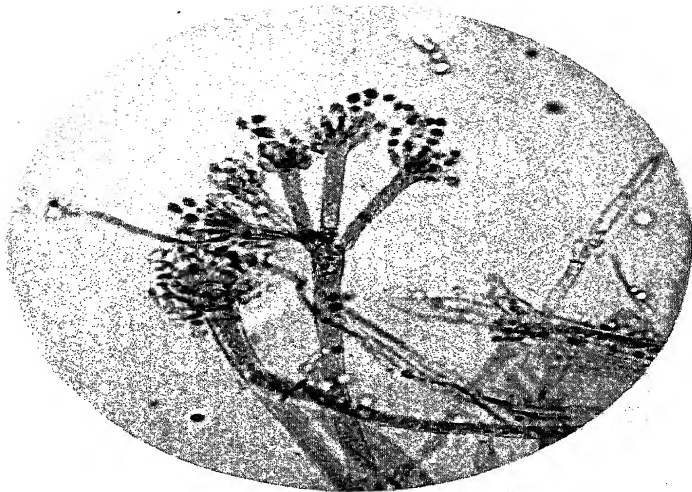


FIG. 69.—*A. glaucus*—proliferation of sterigmata to form secondary heads. $\times 250$.

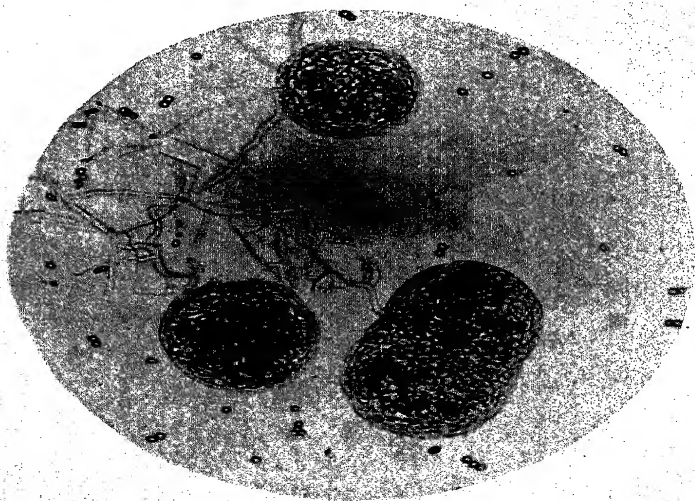


FIG. 70.—*A. ruber*—perithecia. $\times 100$.

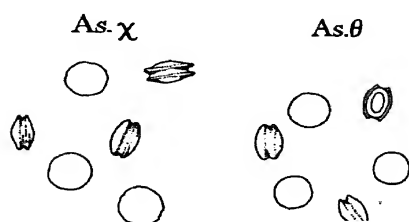
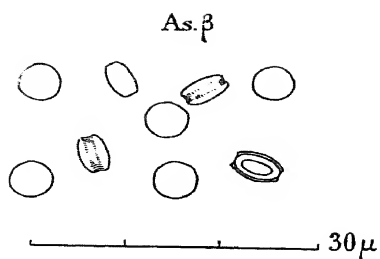


FIG. 71.—Mangin's illustration of various types of ascospore (β = *A. repens*, χ = *A. Chevalieri*, θ = *A. Amstelodami*).



FIG. 72.—*A. restrictus*—columnar heads as seen in Petri dish. $\times 50$.

these the original papers, or the Thom and Church monograph, should be consulted.

Most of the strains of *A. herbariorum*, both series, produce deeply coloured pigments, both on the mycelium and in the substrate, the colours ranging from red to brown or violaceous. *A. ruber* gives a deep ruby-red in the mycelium and a dark red, becoming almost black, in the medium ; it is easily recognized by its almost oblong ascospores without any trace of furrow. *A. repens*, probably the commonest and most cosmopolitan member of the series, gives characteristic colonies, the surface being an intimate mixture of dirty green and yellow, with reverse and medium dirty brown. The name is derived from its habit of producing long trailing or creeping hyphæ, particularly at the shallow end of a slope. *A. Amstelodami* gives colonies of a deep, rich green, speckled with sulphur yellow, and does not colour the medium. *A. Chevalieri* is frequently almost entirely perithecial at temperatures about 25° C. gives an orange-red colour in reverse of colonies and is distinguished by its very small, pulley-shaped ascospores.

Occasional strains belonging with *A. herbariorum series minor* produce no perithecia under ordinary conditions, but are readily assigned to the *A. glaucus* group on conidial characters alone. For further identification, perithecia can usually be obtained by growing on a medium containing a very high concentration, 20–30 per cent, of cane sugar.

The A. penicilloides Group. Species belonging in this group are of common occurrence but are easily missed in plates from infected material owing to their very slow rate of growth, even on rich media. Some of them have been described as species of *Penicillium* and certainly bear a striking resemblance to certain of the monoverticillate members of that genus ; hence the name. In the author's experience they are amongst the most resistant of all moulds to the inhibiting effect of anti-septics. They are easy to recognize by their restricted growth and their very long, slender, columnar heads, composed of chains of fairly large, ovate to barrel-shaped, echinulate conidia. They resemble *A. fumigatus* in disposition of stigmata and shape of heads, and the *A. glaucus* series in size and markings of spores.

Several species have been described, but recognition of their

importance has been tardy and no one has yet made a really adequate study of the group. For the time being, the following species may be distinguished :

- Young colonies pale bluish green, spore chains forming compact columns, conidia slightly roughened, $4-5\mu$ by 3μ *A. gracilis* Bainier.
- Young colonies dark green, heads globose when young, becoming columnar, conidia very dark coloured, $3.5-5\mu$ by about 3.5μ *A. penicilloides* Spegazzini.
- Young colonies dull green, heads in long compact columns, conidia hyaline and cylindrical when young, becoming pyriform and brownish green, $4.5-6\mu$ by $3-4\mu$ *A. restrictus* G. Smith.
- Young colonies dark green, heads more or less columnar, enveloped in slime, conidia $4-6\mu$ by $3-3.5\mu$ *A. conicus* Blockwitz.

Figs. 72, 73 and 74 show *A. restrictus*, a form which has been received from a number of workers in different parts of the world and which appears to be fairly common.

A. fumigatus Fresenius. Colonies are dark, smoky green, often becoming almost black, more or less velvety, or definitely floccose in some strains ; conidial heads columnar, varying in length and about 40μ broad. (Fig. 75). Heads mounted in lacto-phenol have a very characteristic appearance (Fig. 76). The species is readily distinguished from the last group by its globose conidia, $2.5-3\mu$ in diameter, and by its much more rapid growth. A number of perithecial strains, with the conidial morphology of *A. fumigatus*, have been described but are not of common occurrence.

A. clavatus Desmazières. Colonies grow rapidly, forming a dense felt, greyish green in colour. The most typical feature of the species is the elongated, club-shaped vesicle, bearing short, densely packed sterigmata in one series over its whole surface (Fig. 77). It is sometimes possible to find, in ordinary cultures, mature heads which are definitely clavate, but mostly the chains of conidia form dense masses of irregular shape. Large club-shaped heads, on coarse stalks, can be obtained by

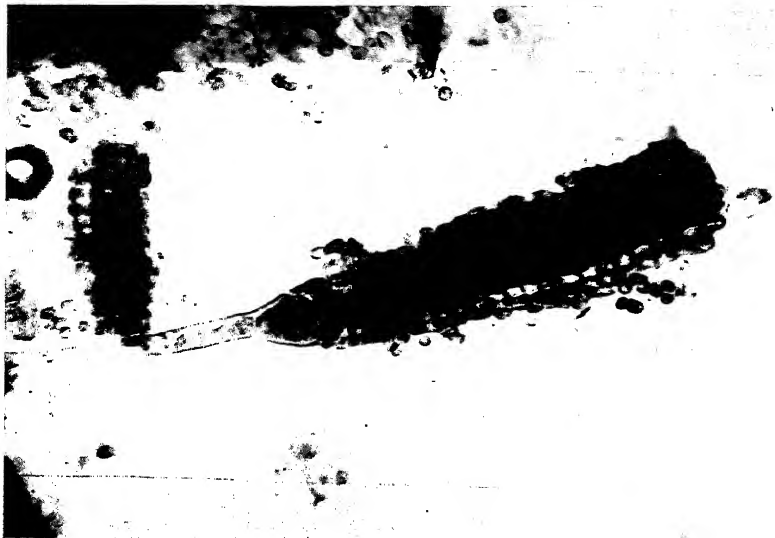


FIG. 73.—*A. restrictus*—typical conidiophore. $\times 500$.



FIG. 74.—*A. restrictus*—spores, mostly young but with a few older ones showing roughening. $\times 1000$.

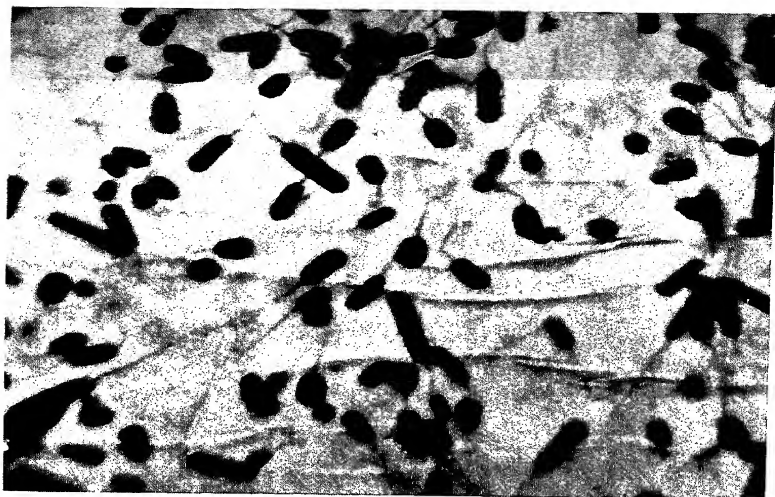


FIG. 75.—*A. fumigatus*—columnar heads as seen in Petri dish. $\times 50$.

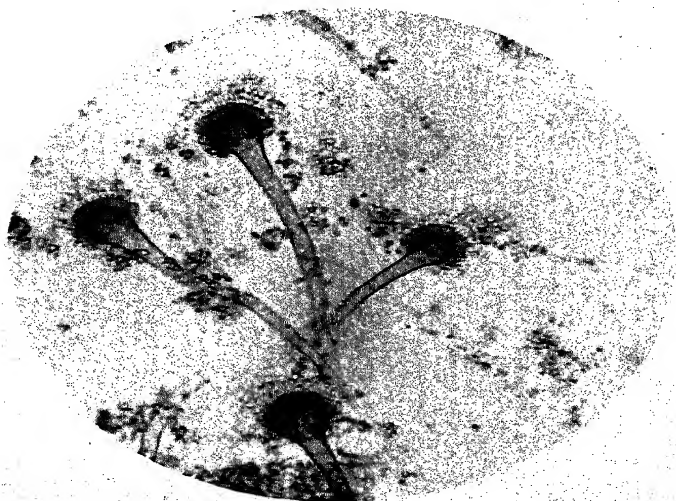


FIG. 76.—*A. fumigatus*—typical conidiophores. $\times 250$.

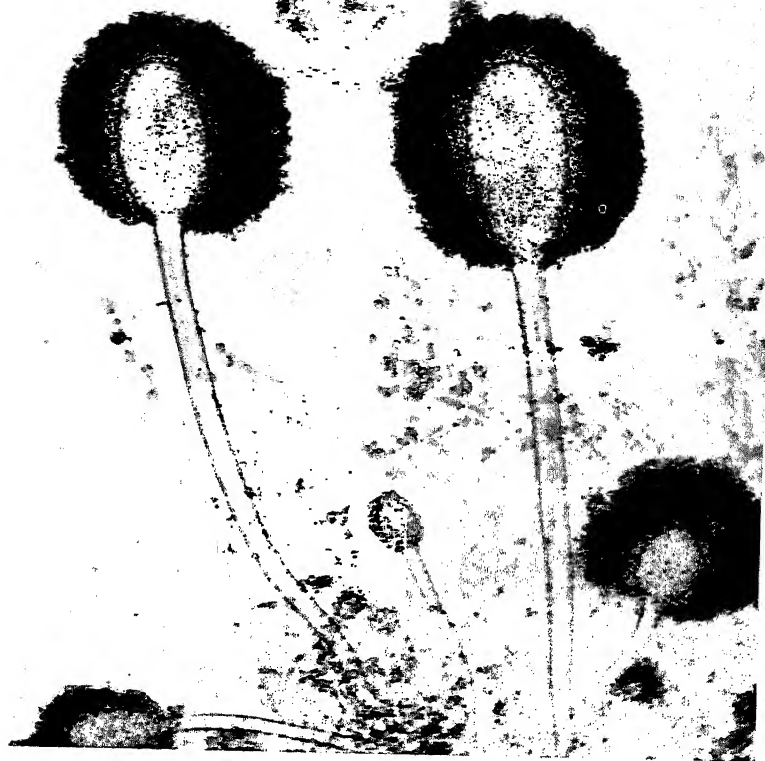


FIG. 77.—*A. clavatus*—conidiophores. $\times 100$.

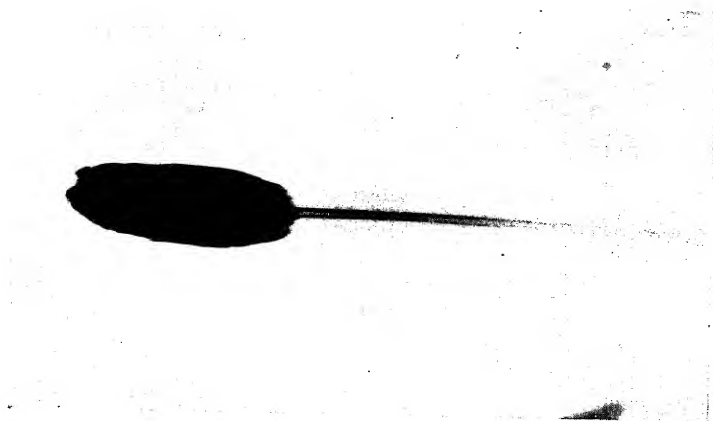


FIG. 78.—*A. giganteus*—single head of characteristic shape as seen in living culture. $\times 25$.

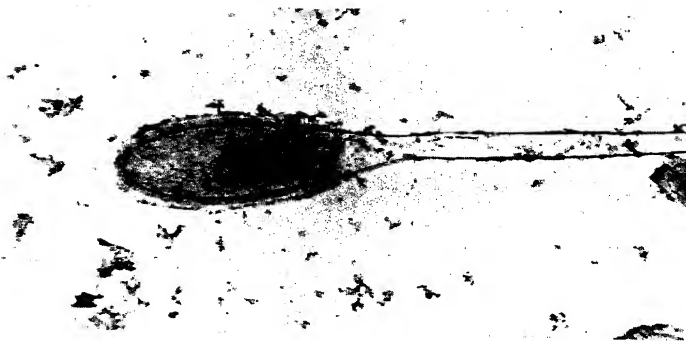


FIG. 79.—*A. giganteus*—heads with clavate vesicles. $\times 100$.



FIG. 80.—*A. giganteus*—heads with elongate vesicles. $\times 100$.

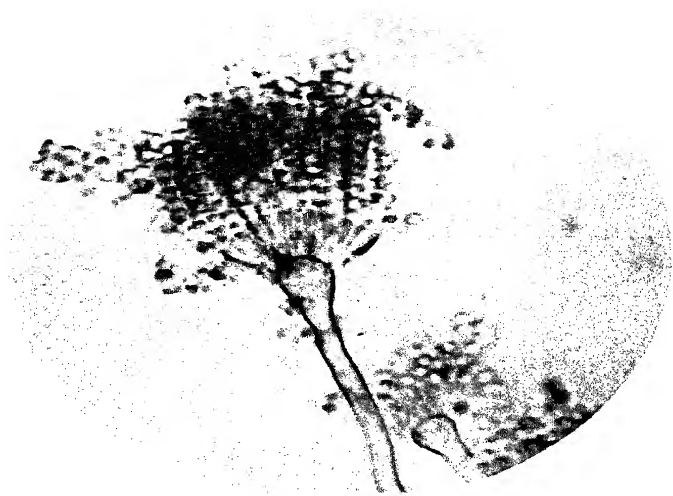


FIG. 81.—*A. nidulans*—head with columnar mass of spores (the spores have remained *in situ* much better than in most mounted specimens). $\times 250$

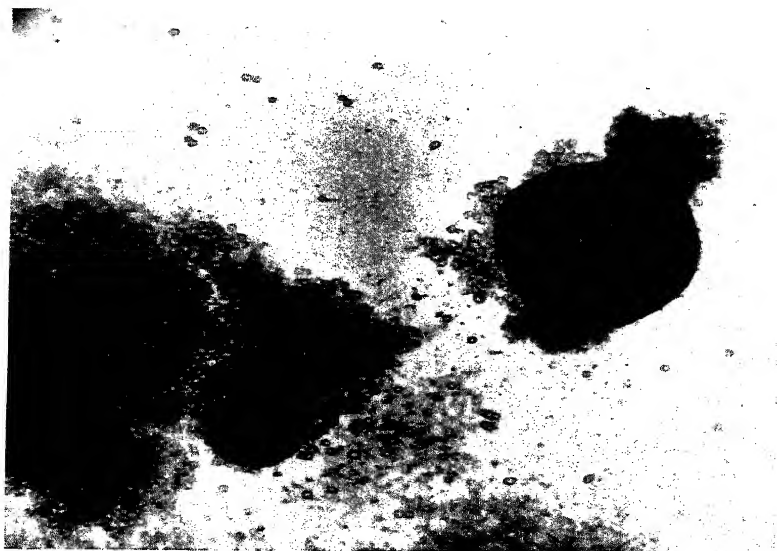


FIG. 82.—*A. nidulans*—perithecia with masses of Hülle cells. $\times 50$.

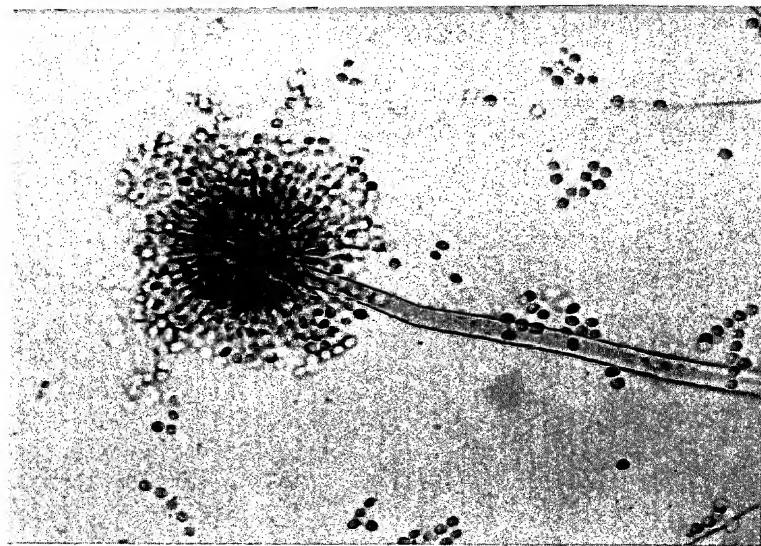


FIG. 83.—*A. versicolor*—typical head. $\times 500$.

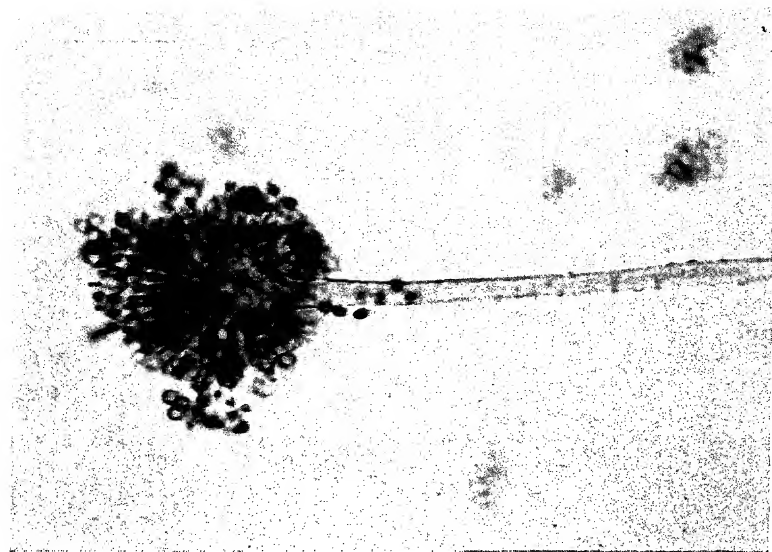


FIG. 84.—*A. Sydowi*. $\times 500$.

growing on special media. The conidia are elliptical, $3.5-4.5\mu$ by $2.5-3\mu$.

A closely related species, *A. giganteus* Wehmer (Figs. 78, 79, 80), differs little from *A. clavatus* when grown on ordinary media but produces enormous heads, on stalks several centimetres long, under special conditions of culture. Thom and Church specify organic nitrogen in the medium whilst Biourge (1933) states that typical gigantic heads are formed only when the medium contains zinc and the cultures are exposed to light.

A. nidulans (Eidam) Winter. Two types of culture are met with, the difference depending on the presence or absence of perithecia. Purely conidial strains form smooth, velvety colonies of a beautiful clear green colour, with reverse deep red to purple. When perithecia are being produced the green colour is confined to small areas, the rest being dirty white and floccose. The conidial heads are similar to those of *A. fumigatus* except for the presence of two series of sterigmata (Fig. 81), with stalks mostly brownish and sinuous. Reduced heads are common, consisting of very small vesicles and few sterigmata, or even single sterigmata on short stalks. Production of perithecia is uncertain and spasmodic. Some strains produce the perfect stage with fair regularity, others are invariably conidial, whilst still others give the imperfect form alone through a series of cultures and then suddenly, and for no apparent reason, produce abundant perithecia. The perithecia (Fig. 82) are more or less globose, dark red, with brittle walls, and are surrounded by masses of thick-walled cells, known as Hülle cells, up to 25μ in diameter. The ascospores are red to purple, ovate, about 5μ by 4μ .

A. versicolor (Vuillemin) Tiraboschi. As the name implies, cultures of this common organism show a considerable range of colour. Different strains may be variously pale green, greyish green, buff, dirty white or even pink in small areas, whilst a single culture, on occasion, may show patches of yellow, pink, white and green on the surface, and a deep red or plum colour in reverse. The texture of the colony may be velvety, floccose, or both in patches. The heads are loosely radiate, with sterigmata in two series and small, globose, delicately roughened conidia (Fig. 83). Whatever the colour of the colony, the spores turn a beautiful emerald green when mounted in lacto-phenol.

A. Sydowi (Bainier and Sartory) Thom and Church. This species is very similar in structure to *A. versicolor* (see Fig. 84), but forms velvety colonies of a deep bluish green or greenish blue colour, with reverse usually very deep red. It is easily mistaken for a *Penicillium* at first sight and until examined under the microscope. Dwarfed and reduced heads are frequently found.

A. terreus Thom. The usual colony colour is sand brown and the surface may be smooth velvety or moderately floccose. The medium is rapidly coloured deep yellow, becoming, especially in the presence of zinc, dirty brown. Fig. 85 shows the typical columnar heads as seen in a living culture and Fig. 86, taken from a mounted specimen, the shape of the vesicle and disposition of the sterigmata. *A. terreus* is a common soil organism and is fairly frequently encountered on all types of vegetable material. The author has found it on scores of samples of Egyptian cotton.

A. ustus (Bainier) Thom and Church. Colonies are usually felted or slightly floccose, yellowish brown to olive brown, with heads more or less hemispherical. The stalks are coloured pale brown; the sterigmata in two series; and the conidia globose, $3.5-4\mu$ in diameter, spinulose.

A. flavipes (Bainier and Sartory) Thom and Church. The usual type of colony is white or silvery white, sometimes with patches of pale pink or mauve, with the white colour persistent or changing to pale buff or greyish buff. Some strains produce brown sclerotia. The buff forms, in old culture, often approach the appearance of *A. terreus*, but the two are readily distinguished by examination of young, vigorously growing colonies. The white strains are not likely to be confused with the *A. candidus* group owing to the difference in shape of the heads. Stalks are yellow or brownish yellow; the heads, at least in old cultures, definitely columnar (Fig. 87); the sterigmata in two series; the conidia smooth, globose, $2-3\mu$ in diameter.

A. candidus Link. The name covers a number of somewhat diverse forms with white or creamy white globose heads. Most strains grow slowly, producing scanty mycelium and glistening white heads, varying from about 100μ to 250μ in



FIG 85.—*A. terreus*—columnar heads as seen in Petri dish.
× 50.

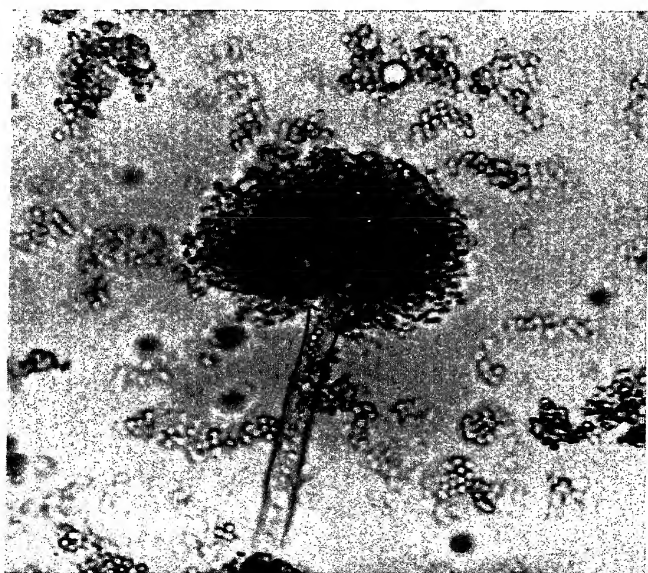


FIG. 86.—*A. terreus*—head with dome-shaped
vesicle. × 500.



FIG. 87.—*A. flavipes*—columnar heads as seen in old culture.
× 50.

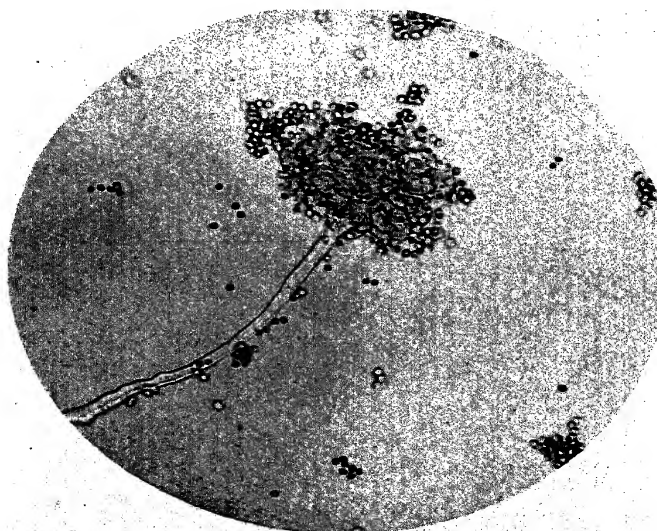


FIG. 88.—*A. candidus*—freak head with swollen cells in
place of normal sterigmata. × 300.

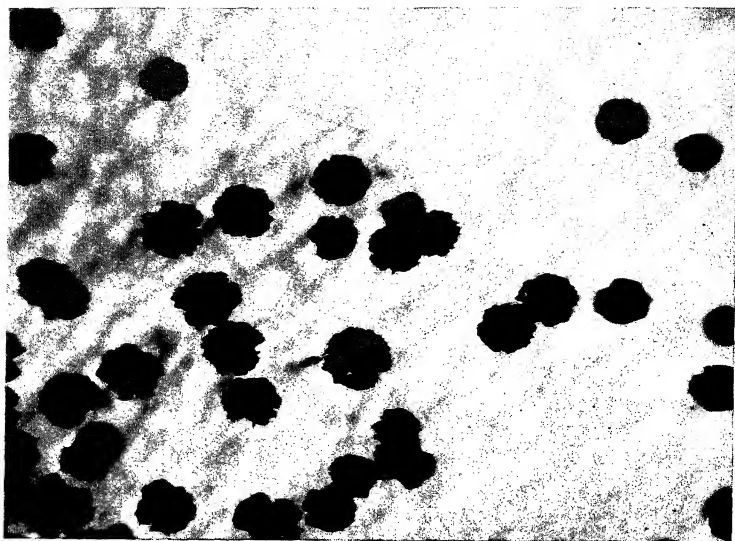


FIG. 89.—*A. niger*—globose heads as seen in Petri dish. $\times 20$.

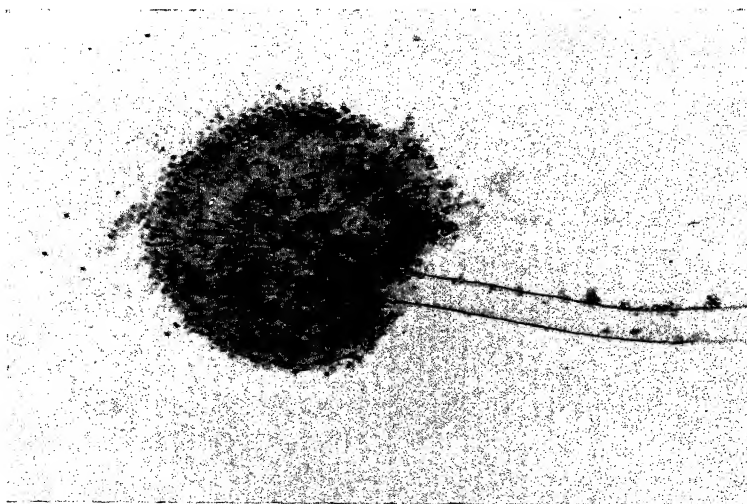


FIG. 90.—*A. niger*—head as seen after bleaching. $\times 250$.

diameter, borne on erect, colourless stalks up to 1 mm. in length. The vesicle is globose and bears two series of sterigmata over the whole surface. Occasionally freak heads are found, with curious swollen cells in place of the usual slender sterigmata (Fig. 88). The spores are globose, smooth, colourless, $2.5-3.5\mu$ in diameter. Some strains produce purple sclerotia, but such are not common.

The A. niger Series. Black *Aspergilli* are of very common occurrence everywhere and, as is usual in any group of widespread distribution and omnivorous habits, there are a large number of races, strains or species. The following group diagnosis will make clear the type of variation to be expected.

Colonies spreading rapidly, with mycelium white at first, frequently developing areas of bright yellow; stalks arising from the substratum, varying from 200μ to several millimetres in length and from about 10μ to 20μ in diameter, mostly colourless, thick-walled, smooth; heads globose, often splitting at the periphery (Fig. 89); vesicles globose, colourless or yellowish brown, fertile over the whole surface, up to about 50μ in diameter; sterigmata in one series in a few strains but usually in two series, with the secondaries fairly uniform in size, about 7μ by $2-3\mu$, and the primaries varying in length from $10-15\mu$ in *A. niger* to 120μ in *A. carbonarius*, both series usually deep brown; conidia globose, brown to black or purplish brown, with the colour aggregated into bars and nodules between the inner and outer walls, appearing spinulose except at very high magnification, varying from 2.5μ to 10μ in diameter. Fig. 90 shows a typical head after decolorization.

The dimensions of primary sterigmata and of conidia are made the basis of separation into a number of fairly definite species. The following table is after Thom and Church, omitting one or two doubtful species.

Sterigmata in one series :

A. luchuensis Inui . . . Conidia $4-5\mu$, sterigmata $7-9\mu$ long.

Sterigmata in two series :

A. luteo-niger (Lutz) T. and C. Conidia about 5μ , with colour bars dissolving in fluid mounts.

A. niger van Tieghem . . . Conidia $2.5-4\mu$, primary ster. $20-30\mu$.

A. phoenicis (Corda) T. and C. . . , $3-4\mu$, , , $40-60\mu$.

A. pulvurulentus (McAlpine)

Thom	Conidia 3-4 μ ,	primary ster.	120 μ .
<i>A. atropurpureus</i> Zimmermann	„ 6-8.5 μ ,	„ „	40 μ .
<i>A. carbonarius</i> (Bainier) Thom	„ 8-10 μ ,	„ „	120 μ .

The species named *A. cinnamomeus* Schieman was obtained as a stable mutant, almost devoid of colour, from *A. niger*.

As indicated earlier, species belonging in this group have interesting biochemical characteristics, and workers who are concerned with this side of their activities will need to separate strains on biochemical, rather than on morphological grounds.

A. Wentii Wehmer. This species is well marked and easy to recognize. The aerial mycelium forms dense floccose masses, white or tinged with pink or rose, completely filling culture tubes when the food supply is abundant. On some natural substrata it is found as a dense, furry, polychromatic growth. The conspicuous spore heads are coffee-coloured, up to 500 μ or even 800 μ in diameter, very ragged-looking when mature, and borne on long slender stalks. The stalks are smooth and colourless; sterigmata in two series with the primaries varying very much in length; and the conidia globose or nearly so, about 5 μ in diameter, roughened or pitted. Fig. 91 shows mature heads in a living culture, Fig. 92 two small portions picked off from a young colony and showing the long smooth stalks, Fig. 93 a specimen mounted in lacto-phenol.

A. ochraceus Wilhelm. The name covers a series of strains which are of common occurrence and wide distribution. Colonies are ochre-coloured or buff. Spore heads are large and conspicuous, at first globose, then splitting into two or more columnar masses, frequently of a shape and colour recalling sheaves of corn (Figs. 94, 95). The stalks are long, usually 10 μ or more in diameter, yellow, and pitted so as to appear definitely rough (see Fig. 96); vesicles globose; sterigmata always in two series; conidia globose or nearly so, smooth or delicately roughened, about 2.5 μ in diameter, occasionally larger. Some strains form abundant heads and no sclerotia; others produce masses of orange to brown sclerotia and comparatively few heads.

A. sulphureus (Fresenius) Thom and Church. A number of strains, which are conveniently grouped as *A. sulphureus*,

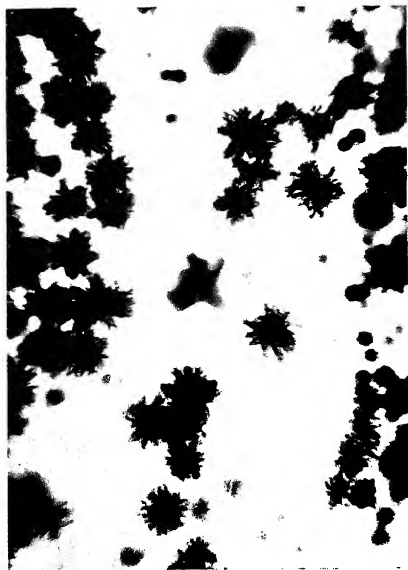


FIG. 91.—*A. Wentii*—mature heads
as seen in Petri dish. $\times 20$.



FIG. 93.—*A. Wentii*—typical head.
 $\times 250$.

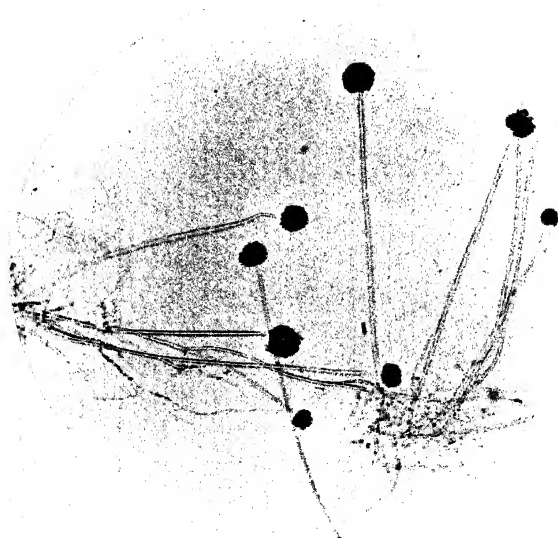


FIG. 92.—*A. Wentii*—portion of young culture (mounted).
 $\times 25$.



FIG. 94.—*A. ochraceus*—heads as seen in Petri dish. $\times 20$.



FIG. 95.—*A. ochraceus*—single large head in old culture, showing characteristic splitting. $\times 25$.



FIG. 96.—*A. ochraceus*—typical head. $\times 500$.

resemble *A. ochraceus* in general aspect and dimensions but differ in that the conidial colour is clear yellow instead of buff. As in *A. ochraceus*, some strains produce abundant sclerotia.

A. tamaritii Kita. The colony colour in mature cultures is brown to deep brown or reddish brown, but young cultures frequently show a distinct greenish tone and have the peculiarly lush appearance of a typical *A. flavus*, indicating a very near relationship to the latter species. The heads are large, at first globose, but becoming loosely radiate, with single chains of spores visible under low magnification. Vesicles are thin-walled and very easily crushed when mounting; sterigmata are typically in two series, but some heads bear one series and it is not unusual to find single and branched sterigmata in the same head; conidia are globose to pyriform, $5-7.5\mu$ in diameter, with nodules of brown colouring matter between the two walls and hence appearing coarsely roughened except under high magnification. *A. tamaritii* resembles *A. flavus-oryzae* in producing kojic acid (see below).

The A. flavus-oryzae Group. Two species are frequently mentioned in the literature, *A. flavus* Link and *A. oryzae* (Alhburg) Cohn, the former yellowish green to true green, and the latter more distinctly yellow and becoming yellowish brown. Examination of numerous strains has shown that there is no sharp line of distinction, and all such races are now usually lumped together as *A. flavus-oryzae*.

Colonies grow rapidly, sometimes definitely floccose, sometimes consisting mainly of closely crowded, erect conidiophores. Stalks are long, mostly $8-15\mu$ in diameter, and usually so densely pitted as to appear spiny. Heads vary much in size and bear one or two series of sterigmata as in *A. tamaritii*. Spores are globose, mostly $3.5-5\mu$ in diameter, occasionally larger, and very rough. Brown sclerotia are known in some strains.

A series of strains of somewhat different appearance from the rest of the group are represented by *A. effusus* Tiraboschi. Young cultures are deeply floccose, white, then dirty greenish yellow, with reverse yellow to rose. Old cultures are dirty brown and have a distinctive cottony appearance. The stalks arise from the aerial mycelium, and it is not unusual to find fertile hyphae consisting of almost uninterrupted series of

foot-cells. The heads otherwise are as in *A. flavus-oryzæ* (Fig. 97).

One of the most interesting features of this group of *Aspergilli* is their power of producing a substance known as "kojic acid." This substance was first obtained by Saito in 1907, from the powdered mycelium of *A. oryzae*. It was later investigated by Yabuta, who showed that it could be obtained by growing the mould on a synthetic medium with glucose as the sole source of carbon, and eventually proved it to be 5-hydroxy-2-hydroxymethyl- γ -pyrone (Yabuta, 1924). Raistrick and co-workers independently discovered kojic acid in 1923, and their work is of great interest in showing that a number of *Aspergilli*, when grown on Czapek's solution, produce kojic acid, and that *all* of these belong in the series represented by *A. flavus*, *A. oryzae* and *A. tamaris* (Birkinshaw *et al.*, 1931). Kojic acid, in water solution, gives a very intense and characteristic blood-red colour with ferric chloride, and this reaction forms a useful diagnostic test for strains in this group. The simplest method of conducting the test is to grow the mould on the liquid medium for any length of time between two and four weeks, filter the solution and add a few drops of a 10 per cent solution of ferric chloride. Raistrick and co-workers grew all their strains on Czapek's solution, but found that the intensity of the reaction varied and that one culture of *A. flavus* gave no reaction. In the author's experience the test is more certain, and the reaction more intense and uniform, if the Raulin-Thom medium is used. *All* strains so far examined give a strongly positive reaction under these conditions.

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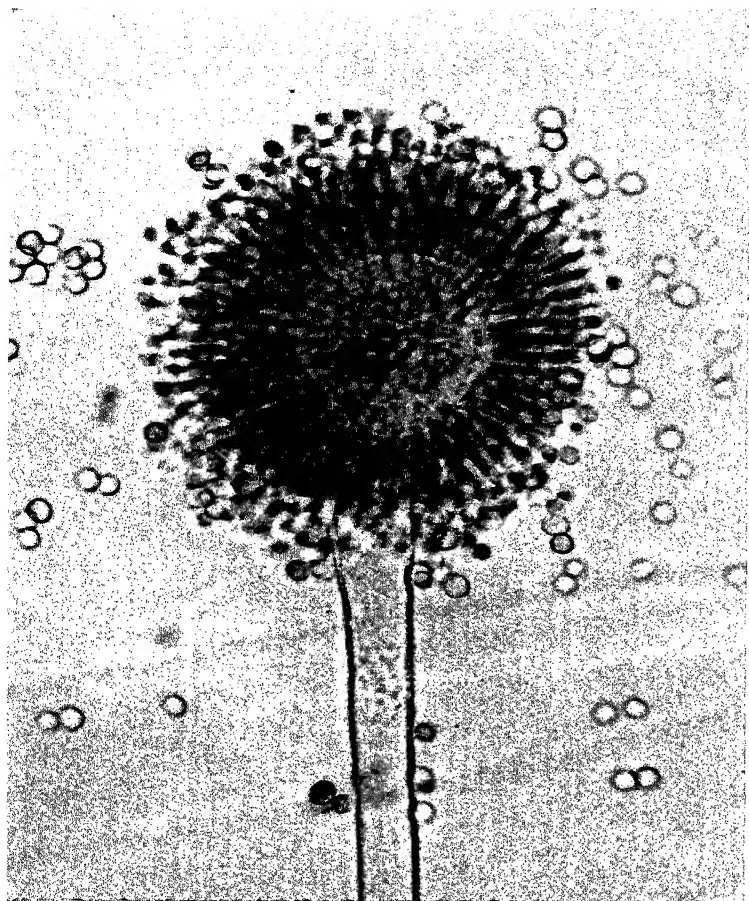


FIG. 97.—*A. effusus*—the type of head commonly seen in slides of any strain of the *A. flavus-oryzæ* series.

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CHAPTER VIII

PENICILLIUM AND RELATED GENERA

The *Penicillia* are closely related to the *Aspergilli* and are just as widespread and omnivorous. Like the *Aspergilli*, many of them are common and serious agents of destruction, but, on the other hand, there are several species which are best known as the means of conducting industrial fermentations. Probably the most important of these are the cheese moulds, the *P. roqueforti* and *P. camemberti* groups. Thom's studies of the occurrence and mode of action of these moulds has made it possible for production of cheeses of the Gorgonzola, Stilton, Roquefort and Camembert types to be carried on anywhere with success, instead of being confined in special regions. The first attempts to produce citric acid commercially by mould fermentation used *Penicillia*, of the group formerly known as *Citromyces*, although modern citric acid manufacture is carried on with the aid of *Aspergillus niger*, owing to the better yields obtained. Comparatively recently a species of *Penicillium* has been used for the large-scale production of gluconic acid. The studies of Raistrick and his school have shown that the *Penicillia* show the most varied metabolic activity, different species being capable of synthesizing from glucose a bewildering variety of substances of complex chemical constitution, and it is conceivable that such work will lay the foundations of future commercial processes employing moulds. Amongst many interesting results already obtained may be mentioned the production of ergosterol, the parent substance of Vitamin D, and of a number of substances closely related to ascorbic acid (Vitamin C).

Unfortunately the taxonomy of the genus presents much greater difficulty than that of the related genus, *Aspergillus*.

There is not the same wide range of conidial colour to serve as a convenient basis of classification, most *Penicillia* being some shade of green during the growing period, and, in addition, the colours are not usually stable but change with varying cultural conditions and with the age of cultures. Further, the number of species of *Penicillium* greatly exceeds the number of *Aspergilli*. The number of specific names which have been bestowed runs into many hundreds and, whilst many of these are synonyms or probable synonyms, there are still about 150 which, in the present state of our knowledge, seem to represent good species.

In view of the difficulties of classification it is not surprising that *Penicillium* has been the subject of several monographs and extensive papers, by Dierckx (1901), Thom (1910), Westling (1911), Sopp (1912), Thom (1915), Biourge (1923), Zaleski (1927) and Thom (1930), to mention only the most important. Thom's latest classification (1930) is the one generally used at the present time, although in some ways it is imperfect and difficult to use. The following is a diagnosis of the genus, embodying Thom's conceptions and introducing several terms which are commonly used in descriptions of species.

Penicillium Link. Mycelium hyaline or pale or brightly coloured, septate, either predominantly submerged or partly submerged and partly aerial, with aerial portion either loose to definitely floccose or partially as ropes of hyphæ; fertile branches (conidiophores) arising from and more or less perpendicular to submerged or aerial hyphæ, either simple or to some degree aggregated into fascicles or compacted into definite coremia, septate, smooth or roughened, terminating in a broom-like whorl of branches (the ***penicillus***), consisting of a single whorl of spore-bearing organs (sterigmata) or twice to several times verticillately branched, with the branching system symmetrical or asymmetrical, the final branches being the sterigmata; conidia produced, as in *Aspergillus*, by abscission, forming unbranched chains, globose ovoid or pyriform, smooth or rough, in most cases green during the growing period; perithecia known in a few species, either sclerotium-like or thin-walled; sclerotia formed by several species and of some diagnostic importance.

Two other terms require explanation. In penicilli with more

than one stage of branching the branches bearing the sterigmata are called *metulae* and the branches supporting the metulae are known as *rami* (Fig. 98).

Langeron (1922) proposed the generic name *Carpenteles* for a species, described by Brefeld as *P. glaucum*, which produces sclerotia which slowly ripen to become thick-walled perithecia, and Shear (1934) has recently revived the name to cover Brefeld's species and a few others discovered within the last few years. Until such time as we have a new monograph on the genus *Penicillium* the question of nomenclature for the ascosporic species may be left *sub judice*.

Thom (1930) recognizes three other genera, closely related to *Penicillium* and including a few very common and important species.

Gliocladium differs from the true *Penicillia* in that a mucilaginous substance is produced by the fruiting organs, and the conidia, instead of standing in detached chains, adhere together or, in the typical species, lose entirely the catenary formation and form solid, slimy balls. The line of separation is not a sharp one, but there are a number of strains in which the distinctive character is well marked and unmistakable.

G. roseum (Link) Bainier is the typical member of a series forming salmon-pink to rose-pink colonies, loosely floccose in texture, with conidia $5-7\mu$ by $3-5\mu$, forming true slime balls somewhat tardily.

G. deliquescens Sopp forms at first *Penicillium*-like colonies of a clear yellow green colour later turning darker and becoming slimy, with conidia mostly $3-3.5\mu$ by $2-2.5\mu$.

G. catenulatum Gilman and Abbott grows as floccose to funiculose colonies, white at first, becoming clear dark green in scattered patches, with conidial chains in long columns, held together by slime, and smooth conidia, $4-7.5\mu$ by $3-4\mu$.

In all the species the penicilli are mostly several times verticillate, with the branches becoming successively more slender (Fig. 99).

Scopulariopsis. The conidial colour is never green as in most of the true *Penicillia*. The penicillus is irregular in form, varying from complex branching systems to single sterigmata produced on short branches from the aerial mycelium. The most distinctive feature is the conidium, which is usually more

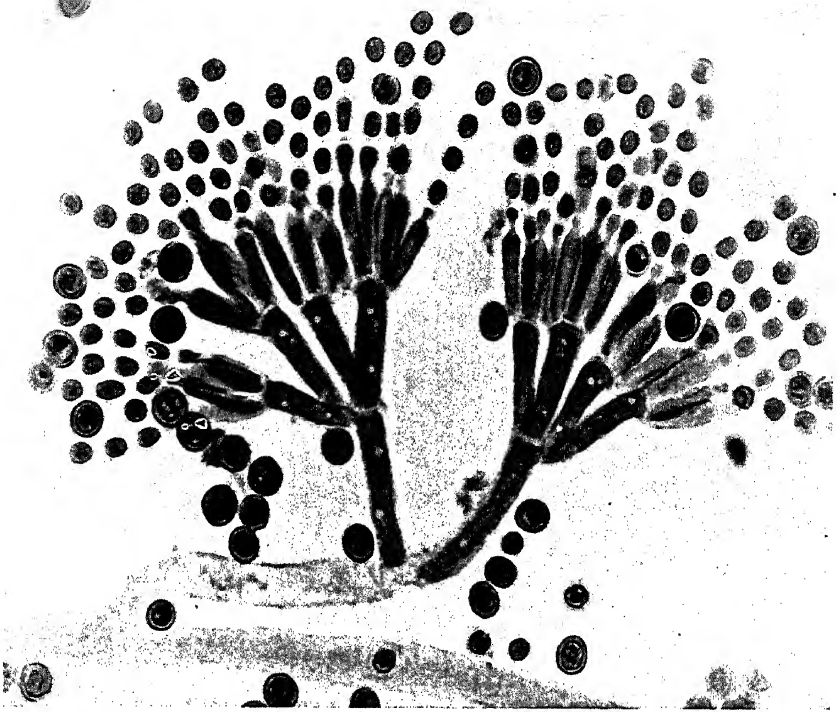


FIG. 98.—*Penicillium*—a typical “penicillus” with several stages of branching and showing the mode of formation of the conidia. $\times 1000$.



FIG. 99.—*Gliocladium roseum*—conidiophores. $\times 250$.

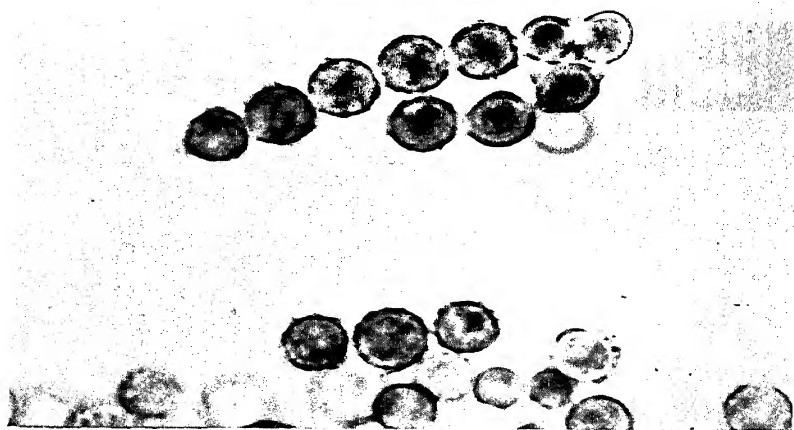


FIG. 100.—*Scopulariopsis brevicaulis*—spores, some showing the typical thickened ring. $\times 1000$.

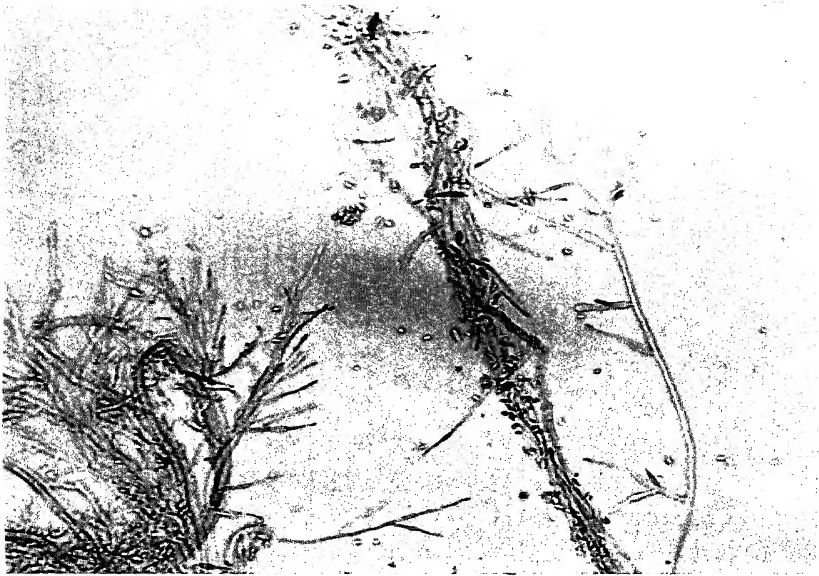


FIG. 101.—*Pæcilomyces varioti*—ropes of hyphæ and various types of spore-bearing structures. $\times 250$.

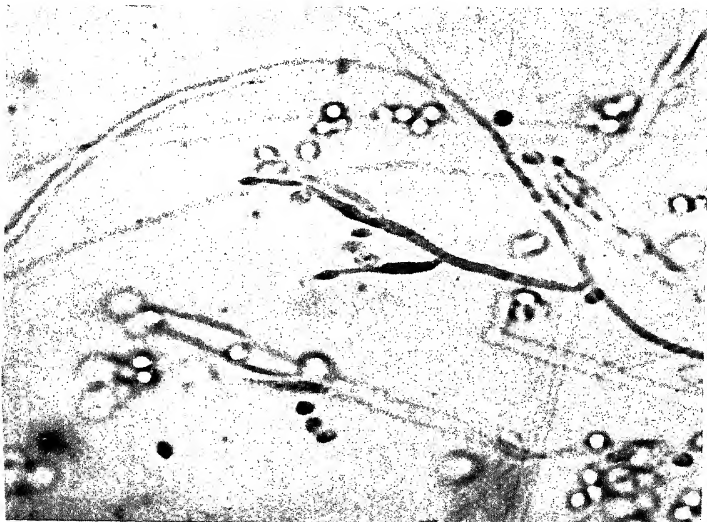


FIG. 102.—*Pæcilomyces* *sp.*—characteristic sterigmata. $\times 800$.

or less lemon-shaped, pointed at one end and at the other having a thickened ring with a central pore (Fig. 100).

S. brevicaulis Bainier is of common occurrence on all kinds of material. It forms brownish, cottony colonies of somewhat thin texture. The conidia are large, $6-7\mu$ in long axis, lemon-shaped, showing well-defined ring and pore and with the outer wall coarsely roughened. A variety, *S. brevicaulis* var. *alba* Thom, is similar except that the colony colour is white and the spores are somewhat larger. A second variety, *S. brevicaulis* var. *glabra* Thom, differs from the parent species in that the spores are smooth; it also forms black sclerotia. A special point of interest is that all species of this genus (and of *Pæcilomyces* too) can liberate arsenic in the form of very poisonous gaseous compounds from any substrate containing even a trace only of this element. When grown on ordinary gelatine, for example, the garlic-like odour common to arsine and its alkyl derivatives is distinctly noticeable. There have been one or two serious cases of arsenic poisoning due to the growth of *S. brevicaulis* on wall-papers coloured with Paris green, and it has been proposed to use this species for detecting minute traces of arsenic in suspected materials, instead of applying the usual chemical methods.

Pæcilomyces. Colony colour varies from white to snuff brown, and the texture is loose cottony or ropy. The penicillus varies much in complexity (much as in *Scopulariopsis*) and the ovate conidia are produced in very long tangled chains. The sterigmata are of very characteristic shape, broad at the base, then tapering sharply to long, slender tips which are usually bent away from the main axis (Figs. 101, 102).

P. varioti Bainier is of widespread occurrence and grows well on almost any kind of substrate. Colonies are pale, dull brown in colour, loosely floccose or funiculose, with spores approximately 6μ by $2.5-3\mu$. It has often been identified as *Spicaria elegans* Harz and there is still some doubt as to whether the latter is a separate species.

The *Penicillia* proper

The multitude of species fall naturally into three main divisions, recognized as a primary basis of separation by all modern authorities. The *Monoverticillata* (to use Thom's term), equivalent to the section *Aspergilloides* of several other

authors and to Wehmer's genus *Citromyces*, have penicilli consisting of a single whorl of sterigmata (Fig. 103). The Biverticillata-Symmetrica have a compact whorl of metulæ, each bearing sterigmata, the whole penicillus being approximately symmetrical about the axis. In addition, the spores are always ovate or pyriform and are borne on slender, tapering (acuminate) sterigmata (Fig. 104). The third and largest group, the Asymmetrica, includes all species in which the penicillus is branched more than once and is asymmetrical, or, if approximately symmetrical, has not the compacted structure and the slender, acuminate sterigmata of the Biverticillata-Symmetrica.

Each large group is sub-divided according to colony characteristics, whether velvety, lanose, funiculose or fasciculate, and it is essential that Thom's conceptions of these terms should be thoroughly understood if his keys are to be used for identifications. The velvety colony has the aerial growth consisting chiefly of conidiophores, arising from submerged hyphæ and giving exactly the effect of a short pile velvet. In lanose species there is a tangled mass of aerial mycelium and the conidiophores arise mainly from these aerial hyphæ, the points of origin being quite away from the substratum. In the Funiculosa part or most of the aerial hyphæ are aggregated into trailing ropes, a character which is usually easy to recognize under a low power of the microscope or even with a good hand lens. Species belonging to the fasciculate sections produce conidiophores, mostly arising from the substratum, which are not evenly distributed, as in the velvety type of colony, but are more or less in clusters, giving a granular or mealy appearance to the colony, or compacted into definite coremia. Thom recommends that colony habit should be studied by cutting thin radial sections from colonies grown in Petri dishes and examining the cut edges of the sections under the microscope. It is, however, usually possible to come to a decision by examination of the edges of the sterile areas which occur between colonies when several colonies are grown in the same dish. Probably the most difficult species to place are those in which fasciculation is slight and whose microscopic appearance is little different from that of the velvety species. In such cases it is often a help if the mould is grown as a single colony on a

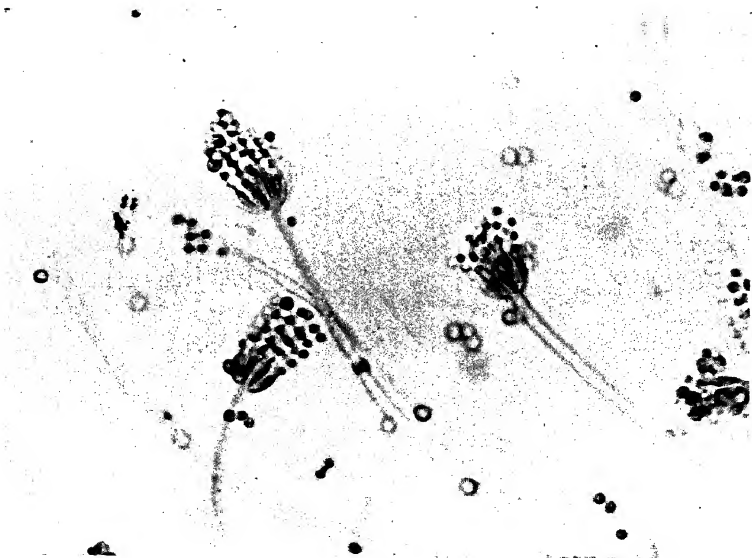


FIG. 103.—*Penicillium spinulosum*—typical monoverticillate conidiophores. $\times 500$.

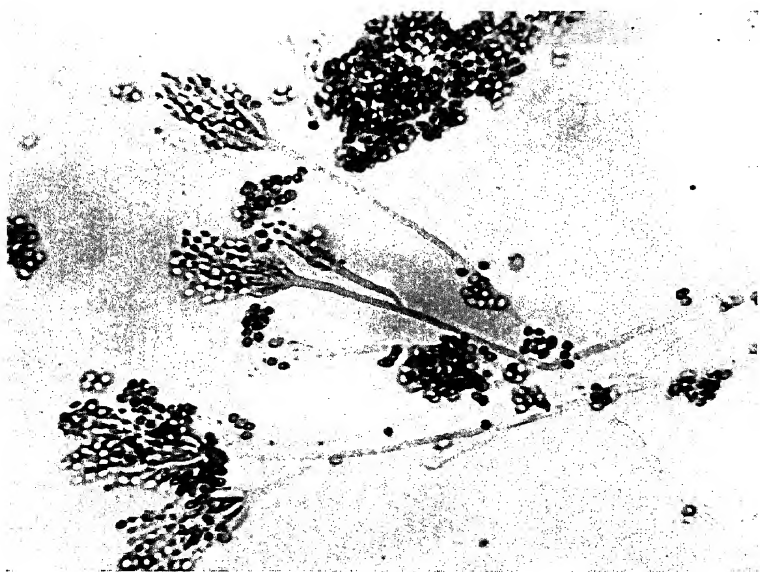


FIG. 104.—*P. luteum*—symmetrically biverticillate conidiophores. $\times 500$.

very thin layer of medium in a Petri dish. Under these conditions some strains of *P. expansum*, for example, will produce concentric rings of small, but definite, coremia when, on slopes, they give colonies which are almost truly velvety.

One of the great difficulties in attempting to identify *Penicillia* is that most of the cosmopolitan species appear to be to some extent unstable. Some species tend to vary in successive cultures made, in the laboratory, under apparently identical conditions but these, with few exceptions, are approximately stable over periods long enough to permit of identification and the instability in question is something more fundamental. With the more common types of organism it is frequently impossible to find an exact fit for a strain in any published descriptions of species, although the strain in question is readily placed as "near to" two, or perhaps three, species previously described. Biourge (1923) gave specific names to all the members of such related series examined by him, but Thom has shown that, when hundreds of strains are examined, differences become less perceptible and the only alternatives are the bestowal of new specific names for almost every strain isolated, and the more practical one of lumping together nearly related strains as a group species. Most living things show, under natural conditions, some tendency to saltation and it is perhaps reasonable to expect that a mould of common and widespread occurrence should be found as a series of slightly different strains, even when such tendency to saltate is not marked. In all the main sections of *Penicillium* there are these group species and, except for the specialist, it is unnecessary to attempt to carry identifications further than the fitting of a particular strain into its group.

In view of the large number of known species it is impossible to describe here more than the best known and common types. The new-comer to a study of this genus is strongly advised to familiarize himself with a number of such forms (by examination of type species, cultures of which can be purchased from any of the well-known culture collections) before attempting to identify unknown strains. This is the most rapid way of acquiring precise conceptions of the diagnostic terms used to define groups and species.



FIGS. 105, 106.—*P. Charlesii*—variously branched conidiophores characteristic of the *Monoverticillata-Ramigena*.
 × 500

The *Stricta-Funiculosa* include a number of species which have been mostly described as occurring in soil. They do not appear to be of any importance in other connections and Thom's book should be consulted for details.

The *Stricta-Velutina* group includes two species of importance.

P. frequentans Westling, synonym *P. glabrum* (Wehmer) Westling, is an exceedingly common type of organism. Although individual strains show considerable variation, there is no difficulty in placing any particular strain in the group species. Growth on all media is very rapid, surface normally a rich green becoming darker and browner in age, with reverse yellow to deep brown. The tip of the conidiophore is swollen and bears a single whorl of sterigmata 9–10 μ long. Conidial chains are packed into very long solid columns (Fig. 107), with conidia globose, mostly smooth, 2.6–3 μ in diameter. When grown on Czapek's solution the liquid, after ten to fourteen days' incubation, gives an intense olive green on addition of ferric chloride, the reaction being due to the formation by the mould of a substance of complex constitution, named citromycetin (Hetherington and Raistrick, 1931a).

P. flavi-dorsum Biourge is typical of a series of closely related strains which appear to be fairly common in this country and which are important because of their abnormal resistance to most antiseptics. Colonies are pale bluish or greyish green with white margin and colourless reverse. Conidial chains are packed in long, solid columns, as in *P. frequentans*, with conidia 3–3.5 μ in diameter, smooth or very slightly roughened.

The Asymmetrica

This is the largest of the three main divisions of *Penicillium*. It is sub-divided into six sections, *Velutina*, *Brevi-compacta*, *Lanata-Typica*, *Lanata-Divaricata*, *Funiculosa* and *Fasciculata*.

The *ASYMMETRICA-VELUTINA* are further sub-divided into seven sections as follows:

Sect. 1. *Elliptica-Magna*. The chief species are *P. oxalicum* Currie and Thom, a soil organism, and *P. digitatum* Saccardo (Syn. *P. olivaceum* Wehmer). The latter occurs chiefly on oranges and other citrus fruits, producing a rot which spreads rapidly and is the cause of serious financial loss in the citrus fruit industry. It can readily be obtained in pure culture from

mouldy oranges on which the infected areas are olive or yellowish green, not blue-green. It grows very poorly on Czapek agar but spreads rapidly on wort agar, producing smooth velvety colonies of a dull yellowish green colour. It is very difficult to prepare good slides showing typical conidiphores as the heads mostly fall to pieces when handled. When unbroken penicilli are found the aptness of the name is evident, for they have a distinct resemblance to a skeleton hand (Figs. 108, 109). All parts of the penicillus are large and the ovate spores, whilst varying much in size, are, on the average, bigger than those of any other *Penicillium*, being mostly 6–8 μ by 4–7 μ , but reaching 12 μ or even more in long axis.

Sect. 2. *Divaricata*. The typical penicillus is biverticillate, but the metulæ tend to spread and each bears a group of short, blunt-pointed sterigmata, the whole disposed so that the penicillus appears as a bunch of monoverticillate heads (Figs. 110, 111). The spores are in a series of divergent solid columns, one column from each metula, giving a very characteristic appearance to colonies as seen under a low magnification (Fig. 112). The only important species is *P. citrinum* Thom, which is of world-wide distribution. Colonies are restricted in growth, velvety, dark bluish grey with a very narrow white edge and reverse varying from very pale to fairly deep yellow. On Czapek's solution and some other liquid media a golden yellow colour is produced. The colouring matter, citrinin, has been isolated by Hetherington and Raistrick (1931b) and its constitution partially elucidated. It is readily obtained as a yellow, crystalline precipitate by acidification of the solution on which the mould has been grown.

Sect. 3. *Radiata*. The name is derived from the habit of growth. Colonies spread broadly and evenly in all directions and thus have always an approximately circular outline. The most important species is *P. chrysogenum* Thom. It has been obtained from a great variety of sources, different strains varying somewhat in cultural and microscopical details but all clearly recognizable as closely related. Colonies are normally widespreading, smooth velvety with overgrowth of white or rosy hyphæ in age, blue-green to bright green when young, turning greyish or purplish brown in age, with broad white margin during the growing period; reverse yellow; transpira-

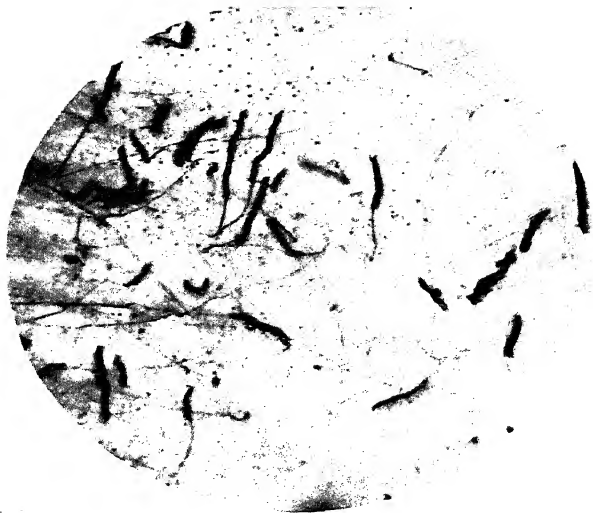


FIG. 107.—*P. frequentans*—columns of spore chains as seen in Petri dish. $\times 50$.

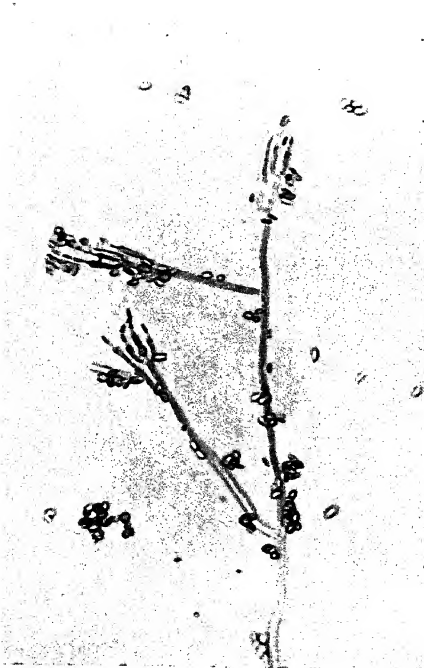


FIG. 108.—*P. digitatum*—conidiophores. $\times 250$.



FIG. 109.—*P. digitatum*—conidiophore. $\times 500$.



FIG. 110.—*P. citrinum*—divaricate penicillus. $\times 500$.



FIG. 111.—*P. Raistrickii*—divaricate penicillus. $\times 500$.



FIG. 112.—*P. Raistrickii*—divergent columns of spore chains as seen in Petri dish culture. $\times 100$.



FIG. 113.—*P. chrysogenum*—penicillus. $\times 500$.

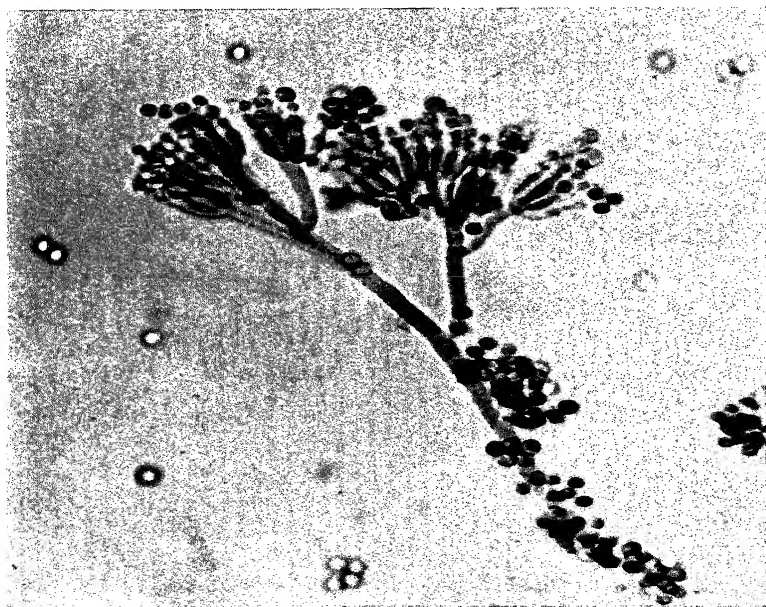


FIG. 114.—*P. roqueforti*—penicillus. $\times 500$.

tion drops abundant, colourless or yellow. The reverse colour varies in intensity in different strains when grown on solid media, but all strains digest milk to give a clear fluid of a rich golden yellow colour. Some strains, when kept in cultivation, tend to produce more and more sterile aerial mycelium, which may mask the characteristic blue-green velvety surface. The penicillus consists of a terminal verticil of metulæ with one or more diverging branches from lower nodes of the conidiophore, with metulæ often more or less divaricate, the chains of conidia being arranged in partially divergent, loosely columnar masses. Metulæ are $10-16\mu$ by $3.5-4\mu$, sterigmata $8-9\mu$ by 3.5μ , and conidia slightly ovate, 4μ by 3.3μ or globose about 4μ in diameter (Fig. 113).

A number of specific names have been given to strains which show slight differences from the type but, for most purposes, these may be ignored. There are a number of strains with all the characteristics of *P. chrysogenum* except that the spores are regularly smaller, about 3μ in diameter instead of 4μ as in the type. Thom suggests that these may be lumped together, with *P. notatum* Westling as type species.

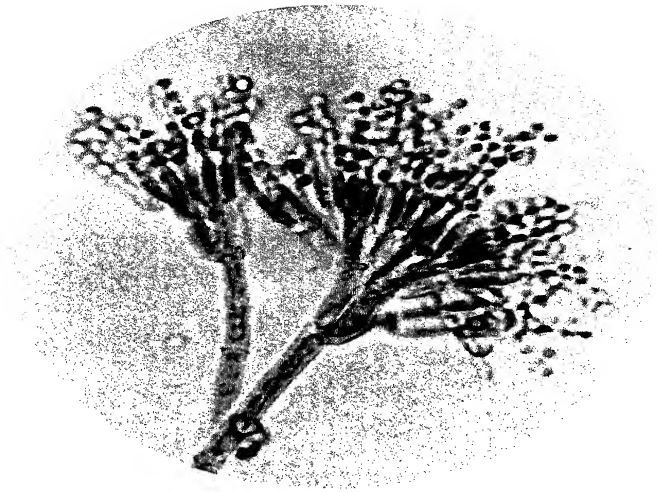
Particular interest attaches to one strain of the *P. notatum* series, isolated by Fleming (1929) and found by him to produce an antibacterial substance named "Penicillin."

Sect. 4. *Restricta*. *P. puberulum* Bainier is representative of a group of forms with similar morphology and differing from *P. chrysogenum* in a more restricted habit of growth and in having more or less roughened conidiophores. Colonies are velvety, becoming slightly zoned in age, blue-green at first, then green persistently, with reverse yellowish or tan and odour sour or mouldy. Conidiophores are usually pitted and the penicillus is more compact than that of the *Radiata*, the heads, as seen in a growing culture, forming fairly dense columns or several slightly divergent columnar masses. Conidia are $3.5-4\mu$ in diameter.

Sect. 5. *Stellata*. The characteristic of species of this group is that they form colonies of a thin, velvety texture, with a broad margin consisting of uneven, radiating lines of conidiophores and described by Thom as "arachnoid" (i.e. like a spider's web). The type and most important species is *P. roqueforti* Thom, the mould used for ripening Roquefort and similar cheeses. Biourge has separated a number of strains

under such names as *P. Stilton* and *P. Gorgonzola*, but Thom has shown that, when a large number of cultures are examined, they are seen to form a homologous series with no sharp lines delimiting separate species. All strains grow well on wort agar, spreading rapidly, with conidial areas a rather bluish green and broad, very uneven margin. On Czapek agar normal colonies are a clear, dark green with reverse varying from colourless to dark green or almost black. Many strains, however, grow very poorly on Czapek agar, giving colonies which are extremely thin and transparent, with very little mycelium and short conidiophores. The usual penicillus is typically asymmetric, with comparatively few elements at each stage of branching and with smooth, globose spores averaging about 5μ in diameter (Fig. 114).

THE BREVI-COMPACTA. The name very aptly describes the penicillus characteristic of this group. There are normally three or even four stages of branching and yet the total length of the penicillus is only 35μ to 45μ at the most (Figs. 115, 116). At the same time metulæ and sterigmata are numerous and closely packed together. Colony texture varies from almost velvety to definitely floccose, but even in the apparently velvety strains (the most usual type) the conidiophores arise, not from submerged hyphæ but from a prostrate, matted layer of surface hyphæ. Colony colour is bluish green or somewhat dirty green, turning to greyish brown in age, with reverse more or less yellow to yellowish brown. All strains liquefy gelatine very rapidly. The type species is *P. brevi-compactum* Dierckx, synonym *P. stoloniferum* Thom. Some five or six other species have been described, but it is questionable whether most of the names are not synonyms of *P. brevi-compactum*. There is often a certain amount of variation between successive cultures of any one strain, the differences frequently being as great as between different so-called species. Occasionally freshly isolated cultures have an *Aspergillus*-like appearance, the conidiophores being long and stiff, with large, complex penicilli and great masses of spores, but these characteristics are seldom retained in successive sub-cultures. Normally the spore chains are twisted and tangled, but examination of very young cultures will often show penicilli which are more or less divaricate, resembling those of *P. citrinum*. The conidiophores are smooth,



115.—*P. brevi-compactum*—normal penicillus.
× 600.

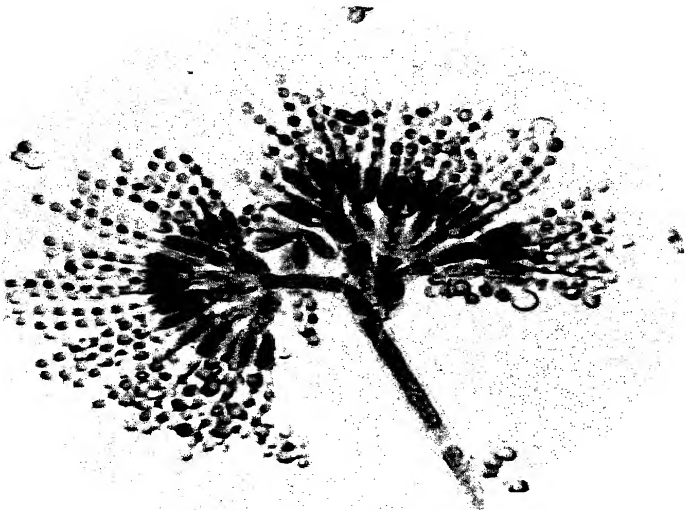


FIG. 116.—*P. brevi-compactum*—very large penicillus.
× 600. Such are not uncommon, particularly in
freshly isolated cultures.

all elements of the penicillus short and the spores are globose or almost so, $2.5-3\mu$ in diameter. *P. brevi-compactum* is of common occurrence and world-wide distribution. It has been collected in many laboratories as an aerial infection; Thom's type strain was found on a rotting mushroom; it has been found on mildewed cotton goods and it is a common infection of stored maize, the consumption of infected grains being supposed at one time to be the cause of Pellagra.

When grown on liquid synthetic media containing glucose, particularly Raulin-Thom solution, a number of phenolic substances are produced (Clutterbuck *et al.*, 1932), detected by adding ferric chloride to the metabolism solution. In the early stages of growth the colour obtained is brown. Later it becomes redder and bluer and, after about three weeks, is an intense bluish crimson. Almost all species in the *P. brevi-compactum* series and, in the author's experience, *all* freshly isolated strains give the characteristic reaction, which is thus a useful confirmation of identification.

THE LANATA-TYPICA. The colony texture in this group is definitely floccose or woolly, at least in young cultures. Colonies usually spread at first as a white felted mass of hyphæ, which slowly becomes coloured as conidial heads ripen, the coloration starting at the centre and spreading outwards. Older colonies may be more or less velvety at the edges and in very old cultures the floccose central areas tend to die down to a compact, felted layer. Spore chains tend to form tangled masses rather than compact columns. The most important species in the group are the moulds used for ripening cheeses of the Camembert, Brie and similar types.

P. camemberti Thom, when grown on laboratory media, forms at first a white, densely floccose colony, slowly becoming pale greyish green. Typical penicilli are long, $70-90\mu$, and comparatively simple, that is with few branches at each stage. The conidia are ellipsoid when young and gradually become globose, $4.5-5.5\mu$ in diameter.

P. caseicolum Bainier, synonym *P. camemberti* var. *Rogeri* Thom, is a pure white form otherwise very similar to *P. camemberti*. It is used in the manufacture of cheeses which differ somewhat in flavour from those made with the true *P. camemberti*.

P. lanosum Westling may be regarded as the type of a series of forms which are fairly often encountered. Colonies are at first white, then grey-green and finally brownish, definitely woolly in texture. The conidia are smooth, or at most very slightly roughened, and not more than 3μ in diameter.

P. biforme Thom has been reported from a variety of sources and seems to be not uncommon. As a contaminant in cheese it is particularly objectionable because of its very strong, mouldy odour. Colonies are greenish grey, of varying degrees of floccosity on different media, often showing densely floccose patches mixed with almost velvety areas. Penicilli usually consist of two rami, with both metulæ and sterigmata few in the verticil and all parts smooth. Conidia are smooth, nearly globose, about 4μ by 3.5μ .

THE LANATA-DIVARICATA. As in the Velutina-Divaricata the penicilli appear as more or less compacted groups of monoverticillate heads, rather than as definite broom-like structures. The floccose character is not so well marked as in the last group, some species being, superficially, almost velvety. In addition, certain species tend to produce funiculose aerial hyphæ, so that there is no sharp line of division between this group and the next. The group includes two series of organisms which are common in soil and hence are found as infections of soil-contaminated materials.

P. janthinellum Biourge is the type of what Thom calls "the soil Penicillia." It is doubtful whether this and related species ought to be in this group at all, as they tend to produce ropes of hyphæ and thus seem to be allied to the *P. terrestre* series (see below). The name refers to the violet colour produced in reverse of colonies, most evident when these occur on plates of soil-extract agar. On Czapek agar the reverse colour is more usually yellow to orange, becoming reddish or faintly violet in age. Penicilli are typically divaricate, but the spore chains are divergent or tangled, not in columns. Sterigmata have long, tapering points and the spores are globose or nearly so, about 2.5μ in diameter, smooth or slightly roughened.

P. canescens Sopp typifies a series of Penicillia which appear to be very common in English soils. Colonies form a thick woolly felt, bluish green or greyish green, with reverse at first yellow, passing to orange and orange-brown. The penicilli

consist of a number of monoverticillate heads arising irregularly along the main conidiophores, bearing fairly compact columns of spore chains. Conidia are smooth, globose or nearly so, about 2.5μ in diameter. The odour is very strong and unpleasant.

P. Janczewskii Zaleski differs from *P. canescens* chiefly in less marked floccosity and in having rough conidia, $3-3.5\mu$ in diameter.

THE ASYMMETRICA-FUNICULOSA. This group includes two series of species of quite different character.

Species in the series represented by *P. Daleæ* Zaleski are closely allied to the *P. Janczewskii* series in cultural and morphological characteristics and, like them, are soil organisms. The main difference is that the *P. Daleæ* series produce definite ropes of hyphæ, usually easily visible under a good hand lens and evident over the whole surface of the colony.

The second series of funiculose species, typified by *P. terrestre* Jensen, differs in that the penicilli are definitely broom-like and not divaricate. Strains allied to *P. terrestre* have been isolated from widely different sources, but there is good reason to believe that the real habitat is soil and that the name is, therefore, an apt one. Colonies are yellowish green to greyish green, floccose, with ropes of hyphæ evident, especially in fairly old cultures; reverse uncoloured or in pale greenish or tan shades. Odour is fairly strong and characteristic, in some strains like moist loam, in others somewhat sour. Penicilli are compact, with conidiophores definitely roughened and smooth globose spores, about 4μ in diameter.

THE FASCICULATA. This is a large group and includes a few species of considerable economic importance. The characteristic of the group is the aggregation of conidiophores into tufts, fascicles or definite coremia. Species which regularly produce coremia are easily recognized as belonging here, and the same may be said of certain strains whose colonies present a rough, granular appearance. There are, however, a fair number of species with a more or less velvety appearance when grown on ordinary laboratory media and which show obvious fasciculation only at the edges of colonies, or only when grown under special conditions or on special media. Most such species show a certain amount of mealiness on the surface of colonies, and

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P. janthinellum Biourge is the type of what Thom calls "the soil Penicillia." It is doubtful whether this and related species ought to be in this group at all, as they tend to produce ropes of hyphæ and thus seem to be allied to the *P. terrestre* series (see below). The name refers to the violet colour produced in reverse of colonies, most evident when these occur on plates of soil-extract agar. On Czapek agar the reverse colour is more usually yellow to orange, becoming reddish or faintly violet in age. Penicilli are typically divaricate, but the spore chains are divergent or tangled, not in columns. Sterigmata have long, tapering points and the spores are globose or nearly so, about 2.5μ in diameter, smooth or slightly roughened.

P. canescens Sopp typifies a series of Penicillia which appear to be very common in English soils. Colonies form a thick woolly felt, bluish green or greyish green, with reverse at first yellow, passing to orange and orange-brown. The penicilli

consist of a number of monoverticillate heads arising irregularly along the main conidiophores, bearing fairly compact columns of spore chains. Conidia are smooth, globose or nearly so, about 2.5μ in diameter. The odour is very strong and unpleasant.

P. Janczewskii Zaleski differs from *P. canescens* chiefly in less marked floccosity and in having rough conidia, $3-3.5\mu$ in diameter.

THE ASYMMETRICA-FUNICULOSA. This group includes two series of species of quite different character.

Species in the series represented by *P. Daleæ* Zaleski are closely allied to the *P. Janczewskii* series in cultural and morphological characteristics and, like them, are soil organisms. The main difference is that the *P. Daleæ* series produce definite ropes of hyphæ, usually easily visible under a good hand lens and evident over the whole surface of the colony.

The second series of funiculose species, typified by *P. terrestre* Jensen, differs in that the penicilli are definitely broom-like and not divaricate. Strains allied to *P. terrestre* have been isolated from widely different sources, but there is good reason to believe that the real habitat is soil and that the name is, therefore, an apt one. Colonies are yellowish green to greyish green, floccose, with ropes of hyphæ evident, especially in fairly old cultures; reverse uncoloured or in pale greenish or tan shades. Odour is fairly strong and characteristic, in some strains like moist loam, in others somewhat sour. Penicilli are compact, with conidiophores definitely roughened and smooth globose spores, about 4μ in diameter.

THE FASCICULATA. This is a large group and includes a few species of considerable economic importance. The characteristic of the group is the aggregation of conidiophores into tufts, fascicles or definite coremia. Species which regularly produce coremia are easily recognized as belonging here, and the same may be said of certain strains whose colonies present a rough, granular appearance. There are, however, a fair number of species with a more or less velvety appearance when grown on ordinary laboratory media and which show obvious fasciculation only at the edges of colonies, or only when grown under special conditions or on special media. Most such species show a certain amount of mealiness on the surface of colonies, and

examination of the edges of growing colonies will always reveal small tufts of conidiophores (Fig. 117).

P. gladioli Machacek is outstanding in this section because of its regular production of sclerotia. When grown at temperatures above 20° C. colonies consist almost entirely of whitish, buff or pinkish sclerotia, produced in concentric rings if the cultures are made in Petri dishes. At about 15° C. the growth is predominantly conidial, with sclerotia scarce. The colonies are then bluish green, with fasciculation evident. This species, as the name implies, is found as a cause of decay in *Gladiolus* corms.

The remaining species are divided into three groups according to their degree of fasciculation.

1. Fascicles and simple conidiophores mixed, but the latter predominating.

2. *Coremiella*. Fascicles and small coremia mixed with simple conidiophores, with fasciculation evident throughout the colony.

3. *Coremia*. Simple conidiophores absent or nearly so. Fructification mainly as definite coremia.

The first group is further divided into “*Aeruginosa*” with blue-green colonies, “*Viridicata*” with pure green or yellow-green colonies, and “*Glaucia*” with dull or greyish green colonies.

P. cyclopium Westling is the commonest of the blue-green species. It is frequently found as a saprophyte and also occurs as a parasite on tulip bulbs, attacking them in the ground and causing distorted growth with blooms misshapen or lacking. On Czapek agar colonies are a rich blue-green, tardily zonate, with fasciculation fairly evident and reverse yellowish or pinkish buff. The conidiophores are definitely roughened whilst the conidia are smooth, globose or nearly so, usually about 3 μ , but occasionally up to 4 μ in diameter.

P. viridicatum Westling gives colonies which are bright green, becoming brown in age, with reverse varying in different strains from yellow to red or orange. Penicilli are complex, with several stages of branching, rough conidiophores and conidia 2.8–3.5 μ or even 4 μ in diameter (Fig. 118).

P. expansum (Link) Thom, the type species of the grey-green section, is best known as the cause of soft rot of apples and pears

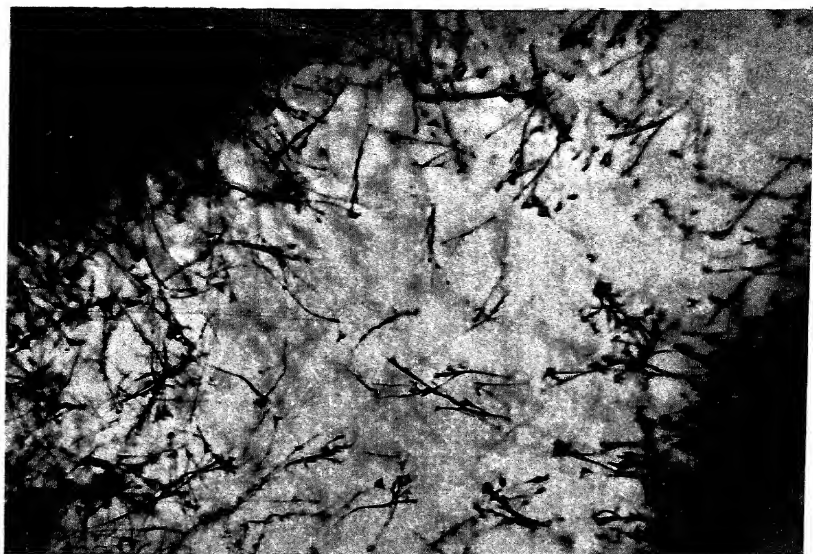


FIG. 117.—*P. brunneo-violaceum*—edge of colony in Petri dish, showing fasciculation. $\times 25$.

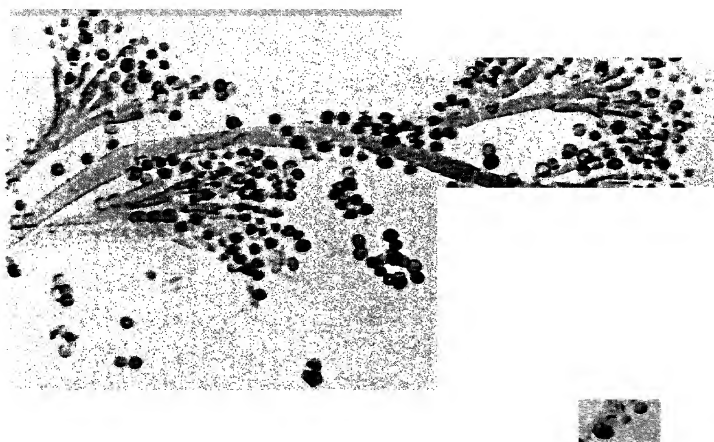


FIG. 118.—*P. viridicatum*—penicillus. $\times 500$. *P. cyclopium* is very similar in appearance.



FIG. 119.—*P. expansum*—single large coremium on agar slope. $\times 25$.

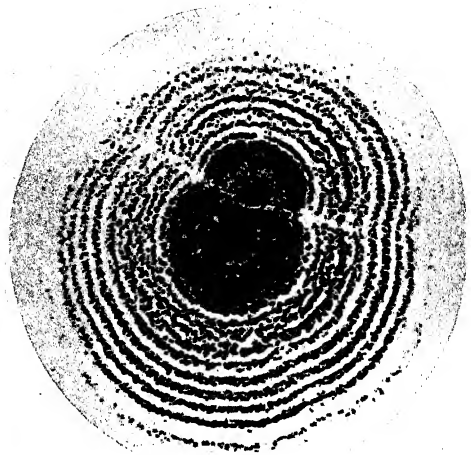


FIG. 120.—*P. expansum*—colony in Petri dish, showing well-marked zonation with rings of small coremia. $\times 0.75$.

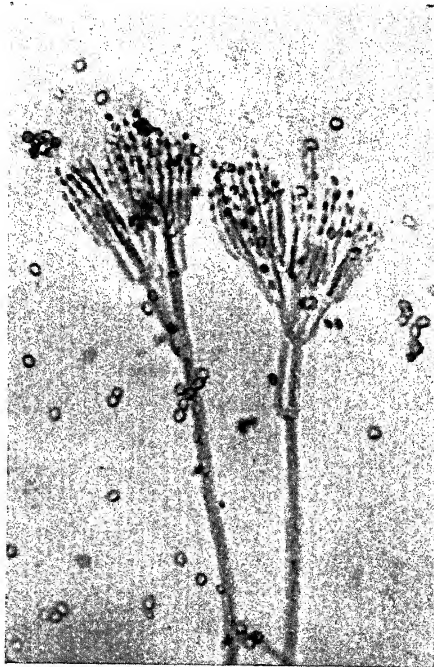


FIG. 121.—*P. expansum*—typical penicilli. $\times 500$.

PENICILLIUM AND RELATED GENERA

in storage, but it is not uncommon on many other ~~surfaces~~. The name covers a large number of strains which vary considerably in the freedom with which they produce coremia and in the degree of zonation of colonies when grown in Petri dishes. Colony colour is green or somewhat bluish green at first, rapidly becoming greyish green and then brown, with a broad white margin during the growing period. Colonies may be granular from the beginning or almost velvety, and showing fasciculation only in age. Fig. 119 shows a large coremium produced in an old culture, on an agar slope, which for the most part was almost truly velvety. The same strain, grown on a thin layer of agar in a Petri dish, is shown in Fig. 120. The concentric rings of coremia, with alternate sterile zones, are readily produced under similar conditions by many strains of this great group-species. The conidiophores, in fluid mounts, have a very characteristic appearance, the metulæ and sterigmata being comparatively few in number but well packed together (Fig. 121). Penicilli are usually $40-45\mu$ in total length. The conidial chains are more or less in columns, the conidia being elliptical when young, 3.3μ by 2μ , becoming almost globose and 3 to 3.4μ in diameter, persisting in chains in fluid mounts.

P. urticae Bainier (= *P. flexuosum* Dale) is of fairly common occurrence and is readily distinguished from *P. expansum*. Colonies are very obviously fasciculate, often forming definite coremia, of a colour which is more grey than green. The penicilli have three or four stages of branching, with all the elements very short, and conidia about 2.8μ in diameter.

In the group Coremiella two species are of importance.

P. corymbiferum Westling is closely related to *P. cyclopium* and shows a similar tendency to parasitize bulbs. Colonies are blue-green to dark olive green, tardily zonate, with broad white margin and reverse deep orange, red or reddish brown, fasciculate all over with numerous small, feathery coremia. A very characteristic feature is the production of respiration drops of a deep orange or ruby red colour. The penicilli are compact, with rough conidiophores and conidia about 3μ in diameter.

P. italicum Wehmer is the cause of serious damage to citrus fruits. It is readily distinguished at sight from *P. digitatum* by the colour of colonies on the fruits, *P. digitatum* being dull

yellowish green and *P. italicum* definitely blue-green. The two species are also distinguished by the type of rot produced. Fruits attacked by *P. digitatum* shrivel and dry up, whilst *P. italicum* produces a soft rot which rapidly reduces the fruit to a slimy pulp. *P. italicum* is also encountered fairly frequently in miscellaneous culture work. It is readily recognized by its production of curious prostrate coremia at the edges of colonies (Fig. 122) and by its characteristic conidiophores, with chains of *Oidium*-like young conidia (Fig. 123). Most strains, when kept in cultivation for long periods, gradually lose the typical colony habit, becoming floccose, with abundant aerial mycelium, and producing coremia only in age if at all.

The Biverticillata-Symmetrica

The name of the division refers to the type of penicillus common to species placed here. In normal cases the conidiophore bears a single whorl of metulæ, each bearing a whorl of sterigmata at approximately the same level, with the whole fructification closely packed (see illustrations). The sterigmata consist of narrow tubes tapering to long points (usually described as acuminate), from which the conidia are cut off as comparatively long, cylindrical segments, becoming and remaining, when ripe, ovate, elliptical or pyriform. The sterigmata and mode of formation of the spores are so distinctive that they serve to place a species in this division even when, as occurs in some strains which fruit very sparingly, the majority of the penicilli do not attain full development and truly biverticillate heads are rare.

There are four sections of the Biverticillata, *Ascogena*, *Coremigena*, *Luteo-virida* and *Miscellanea*.

The ASCOGENA are not of common occurrence and the few species which have been described are of more theoretical than practical interest. The ascocarps are thin-walled, readily broken to liberate the asci, or with scarcely any wall. *P. avellaneum* Thom and Turesson lacks green colour entirely, colonies being of a pale sandy brown colour, with perithecia 300–600 μ in diameter and ascospores ellipsoid, with walls pitted, 6.5–8.5 μ by 4–5 μ . *P. luteum* Zukal and *P. Wortmanni* Klöcker produce yellow ascigeral masses, with ascospores about 4.5 μ by 3 μ , with green or greyish green conidial areas and

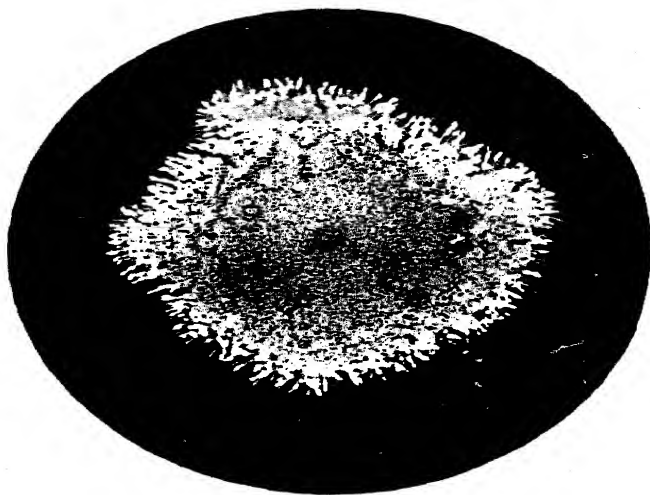


FIG. 122.—*P. italicum*—colony in Petri dish, showing prostrate coremia around the edge. Natural size.



FIG. 123.—*P. italicum*—characteristic penicilli. $\times 500$.
Note the *Oidium*-like young conidia.

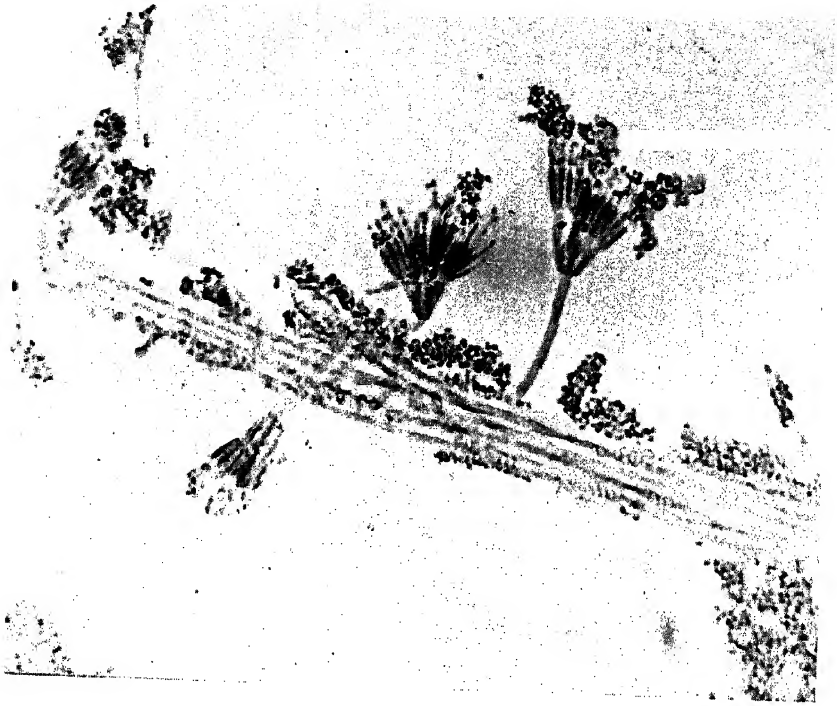


FIG. 124.—*P. funiculosum*—conidiophores arising from hyphal rope.
× 500.

yellow colour in the mycelium. *P. spiculisporum* Lehman has definite perithecia, cream to pinkish, 0.4 to 2 mm. in diameter, and ascospores covered with minute spines.

Of the COREMIGENA only one species, *P. Duclauxi* Delacroix, appears to be at all of widespread distribution. Cultures on media containing cane sugar, such as the ordinary Czapek agar, have a very striking and distinctive appearance. Masses of spiky coremia, several millimetres high, develop early, white at first, then greyish green. The mycelium gradually becomes yellowish whilst the medium slowly passes through yellow to an intense purplish red.

The LUTEO-VIRIDA section includes a number of species of very common occurrence on all kinds of mouldy material.

P. funiculosum Thom is the type of a series of forms which produce ropes of fertile hyphæ, bearing, at right angles, short conidiophores with typically biverticillate heads (Fig. 124). The medium is usually slowly coloured an intense red, but strains are sometimes encountered which reproduce exactly the morphology of Thom's species but lack entirely the red pigment. Conidial colour varies in different strains from pale grey-green to deep green. Conidiophores are 20–80 μ long with sterigmata 10–14 μ , and the conidia are at first cylindrical, then elliptical, 3–4 μ by 2–3 μ .

P. luteo-viride Biourge has colonies of a rich green colour with margin yellow and reverse pink, orange and purplish red in indistinct zones and patches. Conidiophores are short, about 3 μ in diameter, and the conidia ovate, 3–3.5 μ by 2.3–2.5 μ , usually slightly roughened.

P. rugulosum Thom is one of the commonest of all *Penicillia*. Colonies are a deep, rich green, almost velvety, with reverse and edges of colony, but not the medium, deep yellow to bright orange in spots and patches. The conidia are ovate, about 3.5 μ long, and are definitely roughened. An interesting occurrence of this species, and of a number of related strains, some of them approaching closely to *P. minio-luteum* Dierckx, is as parasites of the black *Aspergilli*. Hyphæ of the *Penicillium* twine up the stalks of the *Aspergillus* and smother the black heads with fruiting masses of a dark olive-green colour. Such parasitic forms cause trouble from time to time in factories where citric acid is manufactured by the mould fermentation

process, attacking the mycelial mats of *A. niger* and causing portions to sink in the solution.

P. sanguineum Sopp occurs chiefly as a soil organism. Colonies are of a somewhat yellowish green colour, turning darker and with an overgrowth of yellow to orange hyphæ in age, markedly zonate, with rings of green separated by broad sterile interzones, and with reverse and medium deep orange to blood red. Conidial heads are more or less columnar and dense layers of conidia are formed, breaking off in masses when cultures are disturbed.

P. luteum series. The name covers a series of non-ascosporic forms, of fairly widespread occurrence, in which there is abundant production of floccose, not funiculose, aerial mycelium, more or less yellow in colour, with tardy development of greyish green conidial areas and reverse yellowish to pale rose. The various strains show considerable differences in intensity of colour and freedom of sporing, but they are all supposed to be haplont strains of the heterothallic *P. luteum* (Fig. 104).

P. purpurogenum Stoll shows less luxuriant aerial growth and greater freedom of spore production than the *P. luteum* series. The aerial hyphæ usually pass from yellow to red or purplish red, whilst reverse and medium quickly turn a deep purplish red.

In the section *Miscellanea* Thom groups a number of species with biverticillate penicilli which do not fit into any of the other sections. None of them is of particular importance and some are of very doubtful relationship to the rest of the group.

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CHAPTER IX

LABORATORY EQUIPMENT AND TECHNIQUE

The first essential in any laboratory experimental work involving microfungi is to obtain pure cultures ; that is, to grow each species on a sterile substratum, known as the culture medium, free from admixture with any other organism and with suitable precautions to prevent subsequent contamination. Many fungi fail to grow characteristically in presence of other organisms and identifications are therefore rendered difficult. It is obvious also that studies of nutrition, of metabolic activity, or of the controlling effects of environmental conditions or of antiseptic substances, are without much value unless they are carried out with individual moulds and not with mixtures of species.

Methods of isolation, of culture and of study are, in general, similar to those used by bacteriologists but differ from them in detail. The methods described here are only a selection of those available but will probably be sufficient for preliminary essays in mycological studies, and the advanced worker and specialist can add to them as experience directs.

GENERAL EQUIPMENT

Tubes: Cultures for morphological study and for many other purposes are grown either in tubes or in Petri dishes. The tubes may, for most purposes, be ordinary test-tubes, but the special bacteriological test-tubes are much to be preferred. They are made of glass with a high resistance to chemical action and will withstand repeated sterilization. At the same time they are sufficiently strong mechanically to resist a certain amount of rough handling. The usual sizes are 6" by $\frac{5}{8}$ " and 6" by $\frac{3}{4}$ " or 6" by $\frac{7}{8}$ ". It is an advantage to

have two sizes, the smaller without, and the larger with rims. The rimless tubes pack into baskets and storage boxes much better than the rimmed variety and are used for slopes (see below). The larger tubes with rims are used to hold medium for plating out, when the rim is a distinct help in pouring cleanly and the greater capacity makes thorough mixing of the contents easier.

Plugs : Tubes of culture medium, and tubes containing pure cultures, are always plugged with cotton-wool in order that any air which enters will be filtered from all contaminating organisms. The cotton-wool should be of the non-absorbent variety. It may be obtained in a number of different colours and these are useful for distinguishing the various culture media. A plug should project into the tube about an inch and should have a tuft outside the tube by which it can be extracted, it should fit accurately and tightly, but not so tightly that it cannot be extracted when gripped between any two fingers of one hand, and it should retain its shape, so that, after withdrawal, it can readily be reinserted. There are various methods of making plugs by rolling and shaping before insertion, but an easy and quite satisfactory method is as follows : a strip about $2\frac{1}{2}$ " wide is torn from the sheet of cotton-wool ; from this a rectangular piece is torn, of a size that can be determined only by trial, and the edges are folded in to give a piece $2\frac{1}{2}$ " long and of a width twice the diameter of the tube ; this is laid across the mouth of the tube and its centre gently pushed in by means of a glass rod or the blunt end of an aluminium needle-holder. If the plug is definitely a tight fit when thus pushed in, it will be of the correct fit when the rod is withdrawn. The steaming which it receives during sterilization sets the plug more or less permanently to the shape of the tube.

Rectangular baskets of iron wire, galvanized or tinned, are used to hold tubes during sterilization and may be obtained of any convenient size.

Petri dishes are flat, circular, shallow glass dishes with perpendicular sides, provided with covers of the same shape, but of slightly larger diameter, so that they fit loosely over the dishes. The air which enters, and it must be remembered that fungi require a continual supply of air, is not filtered as in the

case of plugged tubes. Owing, however, to the tortuous path it must take to enter the dish, suspended dust and spores are deposited outside and cultures are reasonably safe from contamination provided the dishes are handled with care and the air of the laboratory is not too heavily charged with infection. As an extra precaution, Petri dishes should, whenever possible, be stored and incubated upside down. Dishes may be obtained in various sizes but, for most purposes, the most convenient size is 10 cm. by 1.5 cm. They are sometimes made with plane, polished bottoms, but this is a refinement too costly for ordinary use.

General Apparatus: A certain amount of the necessary equipment is such as is to be found in any chemical laboratory. This includes beakers and flasks of various sizes, measuring cylinders, pipettes, funnels, Bunsen burners and tripods, balance and weights. It is assumed that the reader is sufficiently familiar with this type of apparatus for it to require no further mention.

A supply of needles and loops will be required for inoculations and the best are made from short lengths of platinum or nichrome wire, permanently fixed into long aluminium handles. Adjustable needle-holders are preferred by some workers, but they have too many hidden surfaces and require much heating to ensure perfect sterilization, particularly when working with liquid media. Occasionally a very stiff needle is required and a convenient form is triangular in section with fairly sharp edges that can be used for scraping. Fine pointed scissors and scalpels are useful for cutting up infected material preparatory to making cultures and the ones made entirely of stainless steel are worth the extra cost, as they stand up to repeated sterilization without corroding. The list of tools should also include three pairs of forceps, one strong and blunt ended, one with fine points, and the third of the special type made for handling cover-glasses.

Sterilization. When handling pure cultures it is necessary to sterilize all tools, utensils and culture media. Metal tools are sterilized by heating in a Bunsen flame, needles until red hot, cutting tools at a somewhat lower temperature to avoid loss of temper. Dry glassware, such as tubes, flasks and Petri dishes, are sterilized by dry heat, and a capacious

air-oven should be available capable of being maintained at a temperature of about 160°C . Three hours' heating at this temperature is sufficient to sterilize anything. Petri dishes are preferably packed in boxes before heating, and allowed to cool and remain therein until required for use. Special boxes of sheet iron or copper are made for the purpose, but they are expensive, and the rectangular tins in which biscuits are sold serve the purpose just as well. Culture media are sterilized by steam, either at atmospheric or higher pressure, so as to avoid change of concentration by evaporation. The Koch type of steamer, designed for sterilization at ordinary pressure, is a tall copper vessel, cylindrical in the usual style, the lower portion of which serves as a water-bath and is fitted with a gauge and sometimes with a constant level attachment. A perforated shelf, fixed a little distance above the water-level, serves to hold apparatus. The lid is provided with a tubulure to take a thermometer and to act as a steam outlet, and the whole outside of the steamer, except the bottom, is lagged with felt. It is normally supplied with a stand of sheet iron and a ring burner. The internal height of the Koch steamer should be sufficient to accommodate a litre flask and a funnel of 7" diameter supported above it. Larger sizes of steamers to hold several baskets of tubes or a number of flasks, are usually rectangular in shape. Where much sterilization of media has to be done it is an advantage to have an autoclave in which steam is generated under pressure, usually 10 to 20 pounds to the square inch, and the time required for sterilization thereby much shortened. Full particulars of autoclaves, as well as of Koch steamers, may be obtained from the catalogues of laboratory furnishers. When heating culture media it is advisable to cover baskets of tubes and the mouths of flasks with grease-proof paper to prevent the plugs being wetted by drips from the lid of the sterilizer. The paper caps should be removed as soon as the vessels are taken out so that the plugs can become thoroughly dry.

Sterile graduated pipettes are frequently required for sowing liquid culture media and for making adjustments to sterile media. They are plugged at the mouth-piece ends, pushing the plugs well into the tubes, wrapped separately in brown paper so that they are completely enclosed, and sterilized by

dry heat, at a temperature not exceeding 130° C. for five to six hours. At a higher temperature the paper is charred and becomes useless as a protection.

Tubes for use in aëration experiments are fitted with the necessary rubber bungs, ready for insertion into flasks or tubes, every open end is plugged with cotton-wool and the whole is wrapped in grease-proof paper and sterilized in the autoclave.

Another matter, which is not strictly concerned with sterilization in the usual sense of the word, may be mentioned here. It is sometimes necessary to sterilize the air of a laboratory when it becomes so heavily charged with infection that cleanly work is difficult. A quick way of partial cleansing of the air is to spray thoroughly with a 2 per cent solution of thymol in spirit, using an instrument which gives a fine, mist-like spray. Thymol is a very efficient fungicide and the alcohol ensures that any spores with which it comes in contact are properly wetted. A thorough method of sterilization, to be used preferably during a week-end, is to charge the air of the room with formaldehyde. A heap of sodium permanganate (potassium permanganate serves as well but is more expensive), is placed on an iron tray on the floor and commercial formalin is poured on in sufficient quantity to wet the heap through. A rapid evolution of gaseous formaldehyde occurs and cleanses the air quickly and efficiently. It is necessary to see that all windows and ventilators are closed before commencing operations and also, when the formalin has been added, to remove oneself quickly, shut the door and see that no one enters the room for at least twenty-four hours. Another good method is to seal up all openings and blow live steam into the room but, obviously, this practice can be adopted only when the fittings of the laboratory are made to withstand it. In some institutions, where the special requirements of microbiological work have been considered at the time of building, the culture laboratory has walls of glazed brick, benches of stone or tiles, and metal doors and window-frames which can be made airtight, the air being steamed periodically as a matter of routine.

Incubators. In order to obtain rapid and characteristic growth of many species of fungi, it is essential to grow them at temperatures above that of the average laboratory, and an incubator, in which a steady, predetermined temperature is

maintained automatically and indefinitely, is absolutely necessary. In physiological work it is often desirable to study the effect of temperature on growth and this, of course, unless the work is to be prolonged unduly, involves the use of a series of incubators set for different temperatures. The optimum temperatures for different fungi cover a wide range, but the great majority of common moulds grow best at about 25° C. In purchasing an incubator for general use it must therefore be specified that the regulating capsule supplied is the correct one for this temperature, since the usual bacteriological incubator is sent out supplied with automatic control for 37° C. It is often better to have two incubators of moderate size rather than one large one so that, if occasion demands, a certain amount of study of temperature relations can be carried out without disturbing cultures which are best kept at 25°.

CULTURE MEDIA

A great many different culture media are used for the propagation and study of fungi, but the majority have been designed for some special purpose, such as the securing of optimum growth of a particular species or for determining the availability of specific substances for mould growth. The only ones which will be noted here are such as are in general use for isolation, propagation and morphological studies. They can be added to or altered as experience suggests.

Mycological media differ in several respects from the media used by the bacteriologist. The latter are usually slightly alkaline in reaction, whereas most fungi prefer a slightly acid medium and many species can tolerate a fairly high acidity. Bacteriological media commonly contain protein as a source of carbon and nitrogen, whilst the majority of the substrates used by the mycologist have carbohydrates as a source of carbon, and nitrogen is supplied in an inorganic form, as nitrates or ammonium salts. The chemical elements which are known to be essential for growth of fungi are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, magnesium and iron. It is probable that, in certain cases, traces of other elements are necessary, but it is seldom that such need to be provided specifically. Many vegetables and vegetable extracts are eminently suitable as culture media without any addition,

or by addition of sugar only, and a few such are very useful for isolation of species and maintenance of stock cultures. Some workers prefer to use purely synthetic media exclusively, as these are of constant composition and therefore strictly comparable when made in different laboratories. There is much to be said for this practice, particularly in taxonomic work. The type of growth shown by any particular species may vary considerably according to the medium used and, in consequence, it is of great importance, when describing new species, to record observations of cultures made on media which can be prepared in identical form by any other worker. In biochemical studies of fungi such synthetic media are used almost exclusively.

Culture media may be either solid or liquid. The term solid media is used in two different senses, to mean either actual solid substances, usually portions of roots, stems or seeds of plants, or aqueous solutions made into jellies by the addition of gelatine or agar. Agar may be considered to be a solidifying agent pure and simple, since it is utilized as a source of carbon by very few species. Its most useful property is the great difference in temperature between its melting-point and solidifying-point. At the concentrations commonly used agar media do not melt till the temperature exceeds 95° C. and can therefore be used for incubating cultures at high temperatures. The molten medium does not resolidify until the temperature falls below 40°, so that infected material or portions of mould growth may be mixed with the medium, before pouring into Petri dishes, without any danger of killing the spores. Gelatine, on the other hand, serves as an excellent culture medium for many moulds without the addition of any other nutritive substance and cannot, therefore, be considered as an inert support when used to solidify any other medium. Gelatine media are readily liquefied on warming and cannot be used at temperatures above 30° at the outside. They are also liquefied by the enzyme action of many fungi, and the chief use of such media is for diagnostic purposes based on the presence or absence of this liquefying property.

Solid media are easier to handle than liquid media and are to be preferred whenever requirements permit. Liquids are used chiefly in biochemical work, when it is often necessary to

determine the course of metabolism by analysis of the medium or to isolate some specific product of fungal activity.

Preparation of Agar Media. Agar-agar is a carbohydrate obtained from certain species of seaweeds. It may be purchased as strips, of a pale brownish colour, or as a fine greyish powder. The strip form is preferred by some workers, as it is said to give a more transparent medium, but it requires prolonged soaking before it can be dissolved, whereas the powder dissolves very readily and is more convenient in use. The amount of agar to use varies with the type of culture fluid. Media which are not strongly acid seldom require more than 1·5 per cent, as this amount gives a jelly which is firm without being too solid and having little tendency to crack. With media which have a distinctly acid reaction it is necessary to use 2 per cent or even more, since agar is readily hydrolysed by heating with acid and thereby rendered incapable of setting.

The method of preparation is very simple if powdered agar is used. The required amount of powder is added to the culture fluid, well distributed by shaking, and the mixture heated to boiling-point. Heating may be carried out in the steamer but is quite safely performed over a Bunsen burner if the liquid is constantly stirred to prevent sticking on the bottom of the vessel. The agar is completely dissolved by the time the temperature reaches boiling-point. For most purposes it is unnecessary to filter the agar medium, the slight cloudiness, which is nearly always present, being no disadvantage, but for some purposes, such as making single spore cultures, involving microscopical examination of agar plates, it is undoubtedly preferable to have a perfectly clean medium. Filtration through ordinary filter paper is impracticably slow, even with the aid of a hot-water funnel or by carrying out the filtration in the Koch sterilizer. The special "Chardin" type of paper, a very thick, soft variety, permits of more rapid filtration but does not give a perfectly clear medium. The best method is to tear up sheets of "Chardin" paper into small pieces, place these in very hot water and shake vigorously until a pulp is obtained, pour this on to a Buchner funnel and suck dry to form a fairly thick pad, wash with hot water until the funnel and flask are heated through, and immediately filter the hot medium with suction. Filtration is very rapid and a

beautifully clean product results. When the agar is dissolved, and after filtration if this is carried out, the medium is filled into tubes, which are then plugged and sterilized, either by autoclaving for twenty to thirty minutes at 15 pounds' pressure, or by steaming for an hour on two or three consecutive days.

Preparation of Gelatine Media. A good-quality sheet gelatine—Coignet's "Gold Label"—is the best—is cut into small pieces, covered with the required amount of water or liquid medium and allowed to soak for several hours or overnight. The vessel is then heated until solution is complete and the medium is tubed and sterilized by steaming for thirty minutes on three successive days. Prolonged heating destroys the power of solidification. This process of intermittent sterilization is always used for media which are unable to withstand continuous heating. The theory of the process depends on the fact that vegetative structures are more readily killed by heat than are the spores. The first short cooking destroys most of the vegetative growth but may not kill all the spores. These, however, being in favourable situation for growth, and being already swollen by the moist heat, germinate rapidly. The second heating destroys the new growth and the third day accounts for any spores whose germination has been delayed. The strength of gelatine media needs to be varied somewhat according to the season. A 10 or 12 per cent solution will give a firm jelly in all but very hot weather, and 15 per cent is sufficient for such occasions.

VEGETABLE MEDIA

1. Potato Plugs. Large, healthy potatoes are scrubbed and washed in water. In some laboratories they are next sterilized by soaking for about twelve hours in a 0.1 per cent solution of mercuric chloride or in a dilute permanganate solution but, if the potatoes are sound, this is unnecessary. The tubers are peeled and again washed, and then cylinders, $2\frac{1}{2}$ to 3 inches long, are cut out with a cork borer. The diameter of the plugs should be only very slightly less than the internal diameter of the culture tubes. The cylinders are cut in two, lengthwise and diagonally, and the halves are put into the tubes, with the thick ends downwards and resting on pieces of wet cotton-wool. The latter is to prevent the surface of the

potato from drying out too rapidly. The tubes are plugged and sterilized by autoclaving at 20 pounds' pressure for twenty minutes on two successive days.

Potato plugs are sometimes useful for bringing about sporulation in obstinate cultures but, like other similar media, they have the disadvantage that only the surface of the colony is seen and characteristic features, which are noted when the reverse side of a Petri dish is examined, are missed.

2. Other Solid Vegetables. Carrots may be used as plugs, made in the same way as potato plugs, or may be cut into slices. Beans are used whole, whilst stems of various plants are cut into short lengths. Otherwise, the details of preparation are the same as for the potato medium.

3. Vegetable Decoctions. Extracts of potatoes, beans, carrots and prunes are the decoctions most commonly used. Fifty grams of prunes or dried beans, 100 g. of carrots, or 200 g. of peeled potatoes cut into small pieces are boiled for an hour in 1 litre of water. The liquid is strained through fine muslin, or filtered, and solidified, if required, with 1.5 to 2 per cent of agar. The medium is tubed and autoclaved for twenty to thirty minutes at 15 pounds. The amounts given are average figures and may be varied according to requirements.

The potato, carrot and prune media give good growth of most moulds, but the bean extract lacks carbohydrate and is considerably improved by the addition of 1.5 to 3 per cent of glucose or cane sugar.

4. Wort. Unfermented sweet wort from a brewery is diluted to a specific gravity of 1.05, heated in the autoclave for half an hour at 10 pounds' pressure and filtered hot. If no autoclave is available the wort may be heated for an hour in the steamer but, in this case, it will be found that subsequent sterilization causes a further precipitate to be thrown down, whereas, if the wort is first heated to a temperature above 100° C. and sterilization is carried out in the steamer, this does not occur. Wort-agar is made by the addition of 2 per cent of agar and the medium, being distinctly acid, should not be over-sterilized.

Wort media give very vigorous and characteristic growth of the great majority of species and are very useful for isolation of species and for stock cultures of some of the more delicate

and slow-growing fungi. Some species, however, tend to produce an undue amount of mycelium at the expense of spore-bearing structures, and these are best kept on a less rich medium. A still richer medium, much favoured by some mycologists, is made with 10 per cent of gelatine instead of agar. One interesting feature of wort is that it is very heavily buffered and has no tendency to become alkaline, as many synthetic media do, when the food material is becoming exhausted. Many of the green moulds, *Aspergilli* and *Penicillia*, turn to a dirty grey or brown shade on such media as Czapek-Dox but retain their green colour for months on wort.

SYNTHETIC MEDIA

5. Plain Gelatine. Simply a 10–15 per cent solution of gelatine in water, prepared as described above.

6. Sugared Gelatine. The addition of 1 to 3 per cent of cane sugar, or glucose, to plain gelatine gives a medium which usually induces more vigorous growth than the unsweetened medium. It serves for determination of liquefying power in many species which grow very poorly in the absence of sugar. The method of preparation is obvious.

7. Raulin's Medium. The first attempt to compound a rational, synthetic medium was by Raulin (1869), who analysed the ash of *Aspergillus niger* and, on the basis of his analysis, made a medium which he used for biochemical studies of this species.

Sugar candy . . .	70 gr.
Tartaric acid . . .	4 g.
Ammonium nitrate . . .	4 g.
Potassium carbonate . . .	0.6 g.
Ammonium phosphate . . .	0.6 g.
Magnesium carbonate . . .	0.4 g.
Ammonium sulphate . . .	0.25 g.
Zinc sulphate (crystals) . . .	0.07 g.
Ferrous sulphate (crystals) . . .	0.07 g.
Potassium silicate . . .	0.07 g. •
Distilled water . . .	to 1,500 c.c.

The only difficulty in making up this solution is to dissolve the silicate, but it is hard to see what useful purpose it serves

and it can certainly be omitted without detriment. The reaction is strongly acid, pH 2.9, and it is almost impossible to make an agar medium which will set. Growth of *Aspergillus niger*, for which the medium was designed, and of a few other species is good, but a great many moulds fail to grow characteristically. Raulin's solution is of interest chiefly because it forms the basis of many other media, some of them of great value.

8. Raulin-Thom Solution. Thom and Church, in *The Aspergilli* (1926, p. 40), make a curious mistake in quoting Raulin's medium and the mistake is repeated in Thom's *Penicillia* (1930, p. 36). Although it is mentioned in the text that the solution includes ammonium nitrate, the formula given omits this salt and substitutes ammonium tartrate. The result is an extraordinarily interesting medium for the purpose of biochemical work and one which gives good growth of most common moulds. It is less acid than the original Raulin's medium, the pH being about 3.9.

The medium is not easy to make up, as magnesium tartrate tends to separate and does not readily re-dissolve. The following is a satisfactory method. The tartaric acid, finely powdered, is dissolved in about 500 c.c. of warm water and the $MgCO_3$ is dissolved in this solution. The ammonium tartrate is added and the liquid stirred till it is all in solution. Next the sugar is dissolved in the mixture. The potassium carbonate is dissolved separately in 100 c.c. of water and added to the rest. The ammonium, zinc and ferrous sulphates are also separately dissolved and added. Finally, the phosphate is dissolved in 500 c.c. of water and added to the main solution with constant stirring, and the medium is made up to correct volume. If properly compounded, the medium should be perfectly clear and of a faint yellow colour which becomes somewhat deeper on sterilization.

9. Neutral Raulin-Dierckx Medium. This is another interesting modification of Raulin's medium, designed by Dierckx for use with *Penicillia* and strongly recommended by Biourge (1923, p. 43). It certainly induces an extraordinary range of colour production in species of *Penicillium* and, in addition, often gives typical growth of coremiform species,

in this and other genera, when other media fail. The method of making up the solution is given by Biourge as follows :

1. Dissolve 0.40 g. of MgCO_3 with 0.71 g. of tartaric acid in 100 c.c. of water.
2. In 800–900 c.c. of distilled water dissolve saccharose 46.6 g.; NH_4NO_3 2.66 g.; ammonium phosphate 0.40 g., K_2CO_3 , 0.40 g.; $(\text{NH}_4)_2\text{SO}_4$ 0.16 g.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.04 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.04 g.
3. Add 66.7 c.c. of solution (1) and make up to 1000 c.c.

In a note on another page (p. 37) Biourge says, "Take 0.27 g. MgCO_3 and 0.40 g. tartaric acid. Bring together in a small mortar until clear, then dilute at once considerably to stop crystallization"; the rest of the formula being as above. He adds, "The small amount of precipitate can be ignored if distributed equally in containers." For a solid medium Biourge recommends the addition of 100 g. of gelatine.

10. Czapek's Solution. Raulin's solution and its direct modifications are unnecessarily complicated and do not lend themselves readily to nutritional studies. Czapek's medium is an attempt to supply all the elements necessary for mould growth without duplication. The basal salt solution is here given as modified by Dox (1910) and Thom and Church (1926, p. 39).

Sodium nitrate	2.0 g.
Potassium chloride	0.5 g.
Magnesium sulphate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g.
Ferrous sulphate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.01 g.
Potassium phosphate, K_2HPO_4	1.0 g.
Water	to 1000 c.c.

The basal solution may be used with various amounts of sugar, with different sugars, or with other sources of carbon. The most usual addition for taxonomic work is 30 g. of cane sugar, and for biochemical work 50 g. of glucose. If glucose is used it is necessary to make up the medium without phosphate and add the latter in concentrated solution after sterilization; otherwise the medium becomes brown and turbid on heating. The reaction is neutral and quite an appreciable amount of magnesium phosphate is precipitated, indicating that the medium might with advantage be slightly modified. Many workers make up the solution with acid potassium phosphate, KH_2PO_4 . The reaction is then definitely acid, the *pH* being about 4.2, but even so, there is still a small amount of precipi-

tation on sterilization. With the acid phosphate there is no browning of glucose on heating. The acid medium gives rather better growth of most moulds than the neutral form.

In compounding the medium it is best to dissolve all the salts, except the phosphate, in about half of the water, add the sugar, dissolve the phosphate separately and add to the rest, finally making up to correct volume.

Czapek agar, perhaps the most generally useful of all solid media, is made by the addition of 1.5 per cent of agar. It is recommended by various authors for taxonomic studies, by Thom and Church for *Aspergilli* (1926, p. 39), by Thom for *Penicillia* (1930, p. 42) and by Waksman for Actinomycetes (1931, p. 279).

TYPES OF CULTURES

Cultures which have to be stored are almost invariably made on agar slopes in tubes. For making slopes in tubes of $\frac{5}{8}$ " diameter the amount of agar medium should be 5-6 c.c. The medium is sterilized in the tubes in the ordinary way and, whilst the agar is still hot and molten, the tubes are inclined at such an angle that the medium forms a layer of decreasing thickness from the bottom of the tube to within about an inch of the plug. Laying the tubes on the bench with the plugged ends supported on a glass rod half an inch in diameter gives about the right amount of slope. The object of sloping the agar is to provide a relatively large surface, on which the progress of growth of a colony can be watched far better than on a level surface of agar in a comparatively narrow tube. Incidentally, the varying thickness of the layer of medium often reveals interesting cultural characteristics, the type of growth at the shallow end showing marked differences from that at the deep end.

The method of sowing slopes is to hold the tubes by their lower ends between the thumb and first finger of the left hand, with the plugs on the palm side of the hand and pointing slightly downwards. Three is the maximum number of tubes which can be conveniently held, allowing of two transfers being made in one operation from a tube culture, or three cultures being sown from infected material. A needle is sterilized by heating to redness in a Bunsen flame. Whilst

it is cooling the plugs are removed, one at a time, by the right hand, using a twisting motion, and placed between the other fingers of the left hand so that they are held by their tops only. The Bunsen flame is played round and into the mouths of the tubes, an operation known as "flaming," until the glass is too hot to touch. The needle is then used to pick up a few spores or a fragment of mycelium from the parent culture and this is deposited, as rapidly as possible and without being allowed to come in contact with the hot glass, on to the fresh agar surface. The mouths of the tubes are again flamed and the plugs inserted. Some workers flame the tubes again, after insertion of the plugs, but this is not necessary unless the plugs have been out of the tubes for an appreciable time, and it causes the tops of the plugs to become charred and messy to handle.

When large numbers of cultures of a single species are required the best method, provided the parent culture is sporulating freely, is to use a spore suspension. A small amount of spore material is transferred, with the usual precautions, to about 1 c.c. of sterile water contained in a very small test-tube, the tube is shaken to distribute the spores and is then supported, as nearly horizontal as possible, in such a way that the mouth can be flamed periodically during the sowing. The inoculations are made with a wire loop, a tiny drop of the suspension being transferred to each fresh tube of medium. Loops of standard size, made of either platinum or nichrome wire and either unmounted or fixed in handles, can be purchased but are readily made from the ordinary wire needles.

When sowing on to liquid media in tubes the method is the same except, of course, that the tubes cannot be held with mouths pointing downwards. They should be held as nearly horizontal as possible without getting the liquid on to the hot glass near the mouth, in order that spores of stray moulds cannot fall directly on to the surface of the medium but will fall on the hot glass and be killed.

For morphological studies microfungi are grown in Petri dishes (commonly known as "plates"), as well as on slopes. Dishes planted with single colonies are useful for determining rate of growth and colony characteristics such as zonation and sectoring, whilst plates containing several colonies are more suitable for microscopical examination. Many species form

dense, opaque felts of mycelium so that the only part of an isolated colony which can be examined by transmitted light is the extreme edge, and this usually shows no ripe fruiting structures. In a dish containing several colonies it is usually found that, along the edges where these approach each other, narrow sterile zones are left and spore-bearing organs can be clearly viewed as they hang over these gaps.

The usual 10-cm. Petri dish requires approximately 12 c.c. of medium to give a layer of adequate thickness, and, if comparative cultures of different species are required, the amount of medium per dish should be fairly accurately standardized. The medium is filled into tubes in correct amounts and sterilized therein. Before pouring into plates it should be allowed to cool down to about 45° C., best by leaving the tubes for a few minutes in a water-bath maintained at this temperature, as very hot medium gives off water vapour, which condenses on the cool lid of the dish and then drips back on to the medium. Each tube, as it is lifted from the water-bath, is held in a sloping position whilst the plug is removed and the mouth flamed, and then the medium is poured gently into the dish whilst one edge of the cover is raised as little as is necessary for the purpose. After replacing the lid the dish is gently tilted to spread the medium and then left to stand on a level surface until the agar has set. Wherever possible it is best to store Petri dishes in the inverted position, both before and after sowing, as this minimizes the chance of infection, and when sowing plates, or handling them for examination, every care must be taken to avoid exposure of the medium more than is absolutely necessary, since protection by flaming, as in the case of tubes, is impossible. With dishes in the normal position it is not easy to sow colonies in predetermined positions, the inoculating needle often leaving a trail of spores right across the surface of the medium. If the dish is inverted, then lifted out of its cover, and the needle approached and receded from directly below, it will be found that very few stray colonies will appear. Another method of sowing single colonies is to inoculate with a loopful of a spore suspension.

For the study of some species which produce very small and fragile sporophores the only satisfactory method of making preparations for microscopical examination is by means of

slide cultures. There are two easy ways of making cultures on microscope slides. If the fungus spreads close to the substratum it is grown on a very thin layer of agar spread on the slide. The agar medium must be clean and transparent. A piece of glass rod or, better, a strip of metal not more than 1 cm. in width is bent twice at right angles, in such a way that it will fit into a Petri dish and support a slide clear of the bottom. The dish, with the support and slide in position, is sterilized by dry heat. A drop of melted agar is poured on the centre of the slide whilst the latter is still warm and spread as evenly as possible by means of a bent glass rod. If it is desired to study the germination of spores, a little spore material is mixed with the agar before pouring on to the slide; but if spore production is to be observed, it is better to plant the medium at two or three points. About 10 c.c. of a sterile 20 per cent solution of glycerol is poured in the bottom of the dish. This keeps the thin layer of agar moist, but not wet, as it would become if pure water were used. The slide is sufficiently near to the lid of the dish to allow observation of the progress of growth with a low-power objective; if the height of the support has been properly adjusted a $\frac{2}{3}$ " objective may be used. When ripe spores are being produced the slide is lifted out and placed for a few hours in a similar dish containing a little formalin, or a solution of osmic acid, in order to kill the fungus and partially fix the structures. The slide may be examined dry, as it is, or, if the growth is too decidedly aerial, with a cover-glass laid on very gently. It is often possible, in this way, to make fairly flat preparations without breaking down fragile structures unduly. With some species permanent mounts may be made in lacto-phenol, afterwards cutting away the agar round the cover-glass and sealing with cement (see under Microscopical Methods).

The second way of making slide cultures is described by Henrici (1930, p. 33) and utilizes a shallow cell, built up on the slide with sealing-wax and a large rectangular cover-glass. Agar is run in to about half the depth—the plane of the slide being vertical—and the fungus is planted on the narrow surface thus provided. All stages of growth are readily observed owing to the spread of the mould being confined approximately to one plane and the effect is as if a thin section through a

colony were being examined. With moulds of vigorous and coarse habit, such as many of the Mucoraceæ, a larger cell may be built up on the same lines, using sheets of thin glass, such as old photographic plates, clamped together in pairs with separators made from narrow strips of cardboard. The photograph of *Thamnidium elegans*, Fig. 19, was taken from a culture made in this way.

In biochemical work moulds are often grown on large volumes of liquid culture media, distributed in numerous conical flasks or trays. These are best sown with a spore suspension, using a measured volume for each container. If the particular mould produces abundant spores, and the number of containers to be sown is not too large, the suspension may be made from one or more cultures on ordinary slopes. About 10 c.c. of sterile water or saline (0.9 per cent sodium chloride in water), is poured into the culture tube and the agar surface is scraped with a stiff wire loop or a flattened needle. The liquid is poured out into a sterile flask, the slope is again scraped with a further lot of sterile water or saline, and the second liquid is added to the first. The concentrated suspension may be diluted as required, or the scrapings from several tubes can be added together to give the desired volume. The flasks or trays are sown with this suspension delivered from a sterile graduated pipette, each container receiving the same volume so that the degree of dilution of the medium is uniform and known. When large numbers of flasks are to be sown, or it is desired to give a heavier sowing, it is often convenient to inoculate from cultures made in "Roux bottles" or in one of the many kinds of culture flasks of similar type. Roux bottles are made of thin glass, are roughly rectangular in section and have cylindrical necks without flanges. A one-litre bottle needs about 100 c.c. of agar medium to give a layer of adequate depth and, after sterilization is laid on its broad side for the agar to set. The easiest way of sowing the bottle is to make a spore suspension, from a slope, in about 10 c.c. of water, pour this into the bottle and tilt backwards and forwards until the whole of the agar surface is wetted. The bottle is incubated until the surface is well covered with sporing heads. To make a suspension from this the agar should be scraped twice or three times with 50-c.c. lots of sterile water or saline.

METHODS OF ISOLATION AND PURIFICATION OF MOULDS

The method used to isolate a particular mould from a natural substratum, and to obtain a pure culture, depends somewhat on circumstances. If the fungus is growing more or less luxuriantly, and typical aerial fruiting organs can be clearly seen, it is usually easy, working with a fine, sterile needle and with the aid of a good hand lens or dissecting microscope, to pick off a few spores, or a single spore head, and transfer to a suitable culture medium. Very often a pure culture results from this first transfer. It is seldom, however, that a mould is found growing, under natural conditions, entirely free from other organisms and there is always a danger that direct transfers will carry a contaminant. When this happens to be a slow-growing species its presence may not be detected for some time and, therefore, cultures made in this way must be watched carefully over a period of several weeks, and purified if at any time there is reason to suspect contamination.

In many cases of mould growth on industrial products, such as leather goods, textiles and cereals, the presence of the fungus is betrayed only by a stain or discoloration instead of the more familiar furry growth. Even when the stain is due entirely to the presence of coloured spores, it is difficult, and often impossible, to demonstrate the presence of spore-bearing heads. In such cases direct cultures can seldom be made without introducing gross contamination. A few adventitious spores of a very rapidly growing species, in presence of a much larger number of those of the causal organism, may result in cultures being completely overgrown with a mould which has nothing whatever to do with the damage. With material of this type the best way of isolating the desired organism is by the operation known as "plating out."

Method. A few tubes of agar medium, 10-12 c.c. in each tube, are heated till the agar is melted and then placed in a water-bath maintained at 40-45° C. until required. In the meantime, a small portion of the mouldy material is reduced to as fine a state of sub-division as possible. A few fragments are dropped, by means of sterile forceps, into one of the tubes of medium, taking the usual precautions in handling the tube, the plug is reinserted and the tube is rotated between the palms of the hands to mix up the contents. The tube should

not be shaken in the usual way as this introduces numerous air bubbles which are very persistent in the viscous fluid. The plug is removed, the mouth of the tube flamed and the contents poured into a sterile Petri dish. The medium from another tube is now poured into the first, mixed by rotation with the small amount of agar remaining after pouring the plate, and then poured into a second Petri dish. The contents of a third tube are now poured into the same tube, mixed and poured as before, and this process is continued for a number of plates which can only be determined by experience with the particular material; usually five or six plates are sufficient. As soon as the agar has set the plates are inverted and incubated.

The rationale of the process is simple. Thorough mixing of the infected material with the agar in the first tube serves to disseminate the spores of the fungus, or, in some cases, fragments of mycelium, throughout the melted medium. The small amount of agar left in the tube after pouring contains relatively few spores and these are again distributed throughout a considerable bulk of agar, and so on. Each plate, after the first, will contain only a fraction of the number of spores contained in the previous one and, on incubation, the successive plates will show fewer and fewer colonies. If the spores of a particular mould are very numerous in the infected material they are likely to persist through all the dilutions and give rise to colonies in the later plates, whereas a few purely adventitious spores, which are to be found on almost any material, are eliminated in the first two or three dilutions. The final plate, if the amount of material and the number of plates have been judged correctly, should show not more than nine or ten well-separated colonies at the outside. If these are all alike, a pure culture has been automatically obtained and nothing is required but to make transfers to slopes or fresh dishes as desired. If more than one mould is present, incubation of the plates should be continued only sufficiently long for the colonies to be differentiated, and transfers should be made immediately from any species which it is desired to study or retain. It is advisable to plate out separately each of the moulds isolated, as soon as the transfers are showing spores, in order to check their purity and effect further purification if this is necessary. It is policy, in a great many cases, to plate

out on two or three different media, for an unimportant mould may grow so well on a rich medium, such as wort, that it swamps a slow-growing species, or, on the other hand, an important species may grow very poorly or not at all on a medium such as Czapek agar and be completely missed unless a more suitable medium is used as well.

Note. A slightly different method of making a series of dilution cultures is often advocated. Instead of pouring all the plates from one tube, which is refilled for each plate, a little of the thoroughly mixed contents of the first tube is poured into the second tube and the remainder poured into a dish, a little of the second is poured into the third tube and so on. The method given above is better in two ways, unless an exact dilution ratio is necessary. It ensures an approximately uniform degree of dilution at each stage and each plate receives the same amount of agar, whereas, with the alternative method, the first plate receives too little and the last plate too much agar.

For the purification of an impure culture it is possible, if the contaminant is of slow growth compared with the chief species, to use the hyphal tip method. A single colony is planted in a Petri dish and, when it is about 1 cm. in diameter, a small piece of agar, containing the tip of a primary radiating hypha, is cut out and transferred to fresh medium. Another method is to make a transfer from a single spore head, using a fine needle and working under a good hand lens. The most usual method, however, is by plate dilution cultures, made as described above. It is necessary to use a very small amount of spore material to mix with the first tube of agar and, in cases where the spores are small and happen to be easily wetted, it is advisable to make one or two preliminary dilutions in tubes of sterile water or saline. Many workers prefer to make cultures which are obtained by germination of a single spore, this being an absolute guarantee of purity. It is extremely probable that the colonies on the last of a series of dilution plates are derived from single spores, but it is not absolutely certain, and it is therefore necessary to use one of the special methods, of which there are quite a number.

(1) A series of dilution plates are prepared in the ordinary way, using a clear, filtered agar. The plates are incubated

only for sufficient time for the spores to put out the primary germ-tubes and must, therefore, be examined under the microscope at frequent intervals during the first forty-eight hours. A few spores are found which are just germinated and which are well separated from all other spores. These are marked, whilst the dish is on the stage of the microscope, the agar cut round them with a sterile scalpel or the flattened end of a needle and the tiny pieces of agar transferred to fresh culture medium. The great disadvantage of this method is that the spores do not all lie in one plane and examination of the plates is extremely tedious.

(2) A better method is to flood the surface of the agar in a Petri dish with a dilute spore suspension in sterile water, allow to stand for a few minutes and then pour off the water. It will be found that quite a number of spores have stuck to the agar and, as these lie approximately in one plane, they are fairly easily found. Otherwise the procedure is the same as in (1).

A very convenient tool for cutting out minute blocks of agar enclosing single spores consists of a dummy microscope objective in which the front lens is replaced by a sharp-edged metal tube, about 5 mm. long and 1.5 mm. in diameter (this being approximately the diameter of the field using a $\frac{2}{3}$ " objective and a $\times 10$ eyepiece). This is screwed on to the nosepiece of the microscope in place of one of the objectives, and, if possible, should be fairly accurately centred with the $\frac{2}{3}$ ". The plate is examined with the latter lens and a field is found which shows a single spore. The cutter is swung into position and lowered until it touches the glass at the bottom of the dish. A small circular block is cut out of the agar but is left behind when the cutter is raised and may be re-examined with the $\frac{2}{3}$ " to ensure that all is well. If the cutter is not perfectly centred with the objective it is only necessary to make a few trial cuts, in order to determine the direction of the error, and thereafter to move the dish the determined amount before making the cut. *

(3) A dilute spore suspension is made by shaking vigorously a small amount of spore material in a tube of sterile water or saline. A series of dilutions, in water or saline, are made from this until a suspension is obtained in such dilution that single loopfuls contain usually one, otherwise no spores. This is

determined by spreading a series of loopfuls on a slide and examining under the microscope. Single loopfuls are then transferred to agar plates and the presence of a single spore in each is confirmed by direct examination. It is advisable to mark the positions of the drops on the bottoms of the dishes so that the spores may be readily located after the drops have evaporated, and so that accidental infection may be recognized by its position on the plate. The plates are incubated and in those in which the spores are viable pure cultures are obtained.

(4) A very ingenious method is described by Hansen (1926) which works well with species which have large, coloured spores, such as species of *Alternaria*, *Helminthosporium*, etc., but is difficult to apply to such moulds as *Penicillia* and *Aspergilli*. A dilute spore suspension is made in melted agar medium. This is sucked up into a number of fine glass capillaries, of bore slightly greater than the diameter of the spores, and the medium is allowed to set therein. Microscopic examination of the capillaries should show short lengths containing each a single spore. These are broken off, sterilized externally with alcohol, and planted in fresh medium, when the spores germinate and the germ-tubes emerge from the ends of the capillaries and form typical colonies.

(5) A number of types of micro-manipulator are marketed with which it is possible to pick up a single spore and transfer it to a new substrate, all under a high power of the microscope. Unfortunately these instruments are very expensive and are not worth purchasing unless much more elaborate work, such as cell dissections, is to be attempted.

MICROSCOPIC EQUIPMENT

A microscope is absolutely essential for any work on micro-fungi. The determination of species involves the use of the highest powers and, whilst much good work has been and may be done by skilful manipulation of indifferent equipment, a really good outfit makes for ease, speed and accuracy. The microscope stand is largely a matter of personal opinion and of funds available, the products of any of the well-known manufacturers being entirely reliable. A focusing and centring substage is necessary, and a mechanical stage is desirable, for

any serious work ; even if an inexpensive stand is purchased, it should be seen that the former is fitted and that a mechanical stage and other refinements can be added as required.

The optical equipment should include a low-power objective, $1\frac{1}{2}$ " or 2", of short working distance, so that it can be focused on the surface of a culture tube laid across the stage without lifting the coarse adjustment out of its bearings, a first-class 8-mm. apochromat and a good oil-immersion objective of 2 or 3 mm. focal length. An ordinary $\frac{2}{3}$ " objective is also useful but not essential. The equipment which is always advertised as standard, $\frac{2}{3}$ ", $\frac{1}{6}$ " and $\frac{1}{12}$ " objectives, is not a good investment for anyone contemplating serious work. The 8-mm. lens possesses certain very great advantages over the 4-mm. or $\frac{1}{6}$ ". It has a larger field, it is comparatively insensitive to variations in cover-glass thickness, it can, with the normal length of drawtube, be adjusted to work on uncovered objects whereas the $\frac{1}{6}$ " cannot unless provided with a correction collar of extra wide range of adjustment, and, at equal numerical aperture, it has twice the depth of focus of the $\frac{1}{6}$ ". In the same way the 3-mm. oil immersion has 50 per cent greater depth of focus than the 2 mm. when eyepieced up to give the same magnification and is therefore to be preferred for work on fungi, as we are usually dealing, not with thin uniform sections, but with comparatively thick, whole structures.

As regards eyepieces, the common Huyghenian form is suitable for use with low powers but does not work satisfactorily with apochromats or with high-power achromats. Compensating oculars are made for use with the latter, but there are several special types which are compensated for use with apochromats and have distinct advantages over the old compensating eyepieces in giving a larger and flatter field. Suitable powers are $\times 8$ and $\times 12$; in addition a $\times 20$ ocular can be used to advantage with the 8-mm. apo. A measuring ocular is also essential and, since very great accuracy of particular measurements is seldom required, in view of the considerable normal variations in the dimensions of parts of a fungus, a glass disc micrometer, fitted in an ordinary eyepiece, is quite satisfactory. Under suitable conditions considerable accuracy can be attained with this simple and comparatively inexpensive apparatus, and it is far easier and quicker to use

than the more elaborate filar micrometer. As far as the writer is aware, only one make of eyepiece, the "Periscopic" $\times 15$, is so constructed that a disc micrometer resting on the diaphragm can be calibrated to give values of one division exactly equal to 1μ with the 2-mm. objective, 5μ with the 8-mm. and 10μ with a $\frac{2}{3}$ ". With most oculars the best round figures which can be obtained are, respectively, 1.5μ , 7μ , and 15μ . In the opinion of most microscopists it is preferable, except in very exact work which demands a more accurate type of measuring ocular, to calibrate micrometers at tube lengths which give round figures and to suffer the slight deterioration from perfection of image due to working at a wrong tube length, rather than be obliged to reduce all measurements to normal units by calculation.

As an accessory to a series of good objectives an efficient substage condenser is essential, if reliance is to be placed on observation of fine detail. The Abbe type is commonly used and is easy to manipulate, but a properly corrected achromatic condenser should be selected in preference whenever possible. Most English manufacturers make two forms, of which the one of larger diameter and longer focal length is somewhat easier to use and the better suited to low-power objectives.

No serious work can be done with daylight as an illuminant for the microscope. The ideal source of light is of small area and high and uniform intensity and undoubtedly the best available is the enclosed tungsten arc, typified by the "Pointolite" and "Pointlight" lamps. In order to obtain an image of the light source of reasonable dimensions, for use with objectives of lower power than 2 mm., it is necessary to use a bull's-eye condenser. As pointed out by Coles, a cheap and really efficient bull's eye is the field lens (the larger of the two lenses) from $\times 6$ Huyghenian eyepiece. It is not difficult to mount this on the lamp, with flat side towards the source of light and at a distance therefrom equal to the focal length of the lens. It should, if possible, be arranged to swing in and out from the optical axis so that it is always in perfect alignment when in use. Another suitable electric lamp, of lower intensity than the tungsten arc, is that described by Barnard and Welch. It utilizes an ordinary incandescent lamp and a stout glass rod, polished at one end and finely ground at the

other, as a condenser. The multiple reflections inside the glass rod even out the inequalities in the beam of light and, when the rod is properly centred and focused, the ground end acts as a light source of almost uniform intensity. Where electric current is not available a very convenient gas lamp is the "Thorium Lamp," made by Messrs. James Swift and Sons, in which a small, circular pastille of thorium oxide is made incandescent by means of a flat, non-luminous flame. In spite of the convenience of electricity and gas, many workers still prefer the old-fashioned flat-wick oil lamp. With the flame turned edge on to the microscope it gives a very uniform light and one with which it is pleasant to work.

Whatever equipment the mycologist possesses it is essential, if accurate and reliable information is to be obtained by its use, that the worker should thoroughly master the technique of the instrument. Anyone who is new to microscopical work ought to read and digest one or two of the books recommended.

In addition to the apparatus already described, a good hand lens is very useful, particularly for the preliminary examination of cultures and observations of fungi growing on natural substrata. The best kind is the type known as "Aplanatic." It consists of three lenses cemented together and, although comparatively expensive, is worth the difference in price, compared with the singlets and doublets, in that it gives a much flatter field and is almost entirely free from chromatic aberration.

MICROSCOPICAL METHODS

When examining cultures for diagnostic purposes a great deal of information can be obtained by the study of dry, living cultures under the compound microscope. Slopes can be placed across the stage and the edges examined by transmitted light with objectives of $\frac{2}{3}$ " and lower power. Petri dishes can be laid flat on the stage, either side up, and examined by incident or transmitted light. If the mould forms dense, matted growth, the planting of several colonies in one dish will often result in narrow sterile zones, where the colonies approach each other, and mature fruiting organs can be observed partially overhanging these clear spaces. In the case of *Aspergilli* and *Penicillia* the shape of the fruiting heads, the disposition of the chains of spores and the origin of the coni-

diophores, whether from submerged or aerial hyphæ, have considerable diagnostic value, and the required information can be obtained only from the study of undisturbed, living cultures. In the same way, determination of species of *Mucor* and *Rhizopus* is possible only when study of slide preparations is combined with examination of living material in Petri dishes. Many other moulds have conidial structures which fall to pieces at the least touch and, with these, the value of direct observation is apparent.

For study of fine detail, and for accurate measurements, slide preparations must be made. With most species of moulds it is difficult, if not impossible, to fix, stain and mount specimens, as is done for botanical and zoological material, and at the same time preserve structure. In all but a few cases it is general practice to use fluid mounts prepared with as little manipulation as possible. Water is entirely unsuitable as a mounting medium. It evaporates rapidly, it causes shrinkage of hyphæ by osmosis and it usually causes the parts of the specimen to adhere together as a tangled mass of hyphæ, spores and air bubbles. Alcohol wets efficiently and makes a fairly satisfactory mountant for a brief and rapid examination, but is too volatile for making permanent slides. Easily the most generally useful medium is that known as "lactophenol."

Lactophenol

Phenol, crystals.	2 parts.
Lactic acid, B.P.	2 parts.
Glycerine, pure .	1 part.
Water	2 parts.

It is readily prepared by warming the phenol with the water until dissolved and then adding the lactic acid and glycerine. If the water is substituted by a saturated solution of picric acid or a solution of cotton blue, a combined stain and mounting medium is obtained. Fungal hyphæ, when fairly young, take the stain well when mounted in the coloured fluid and leave the "background" almost colourless. The medium containing picric acid is particularly useful for photography, when a blue light filter may be used to increase contrast. For observation of colours of spores and hyphæ slides must, of course, be made with plain lactophenol. The great advantages

of this mounting medium are that it does not cause shrinkage of the cells of the fungus and that it has no tendency to evaporate, so that permanent preparations are readily obtained.

The method of mounting is to place a *small* drop of mounting fluid in the centre of a clean glass slide, with a sterile needle pick off from the culture a very small portion of typical material, place this in the drop of fluid and very gently tease it out with a pair of needles until it is well wetted, then lower on to it a cover-glass in such a way as to avoid air bubbles as far as possible. It is difficult to make slides which contain no air bubbles without teasing out the specimen to such an extent that structure is destroyed, and within reason, their presence does not seriously interfere with observation. Some moulds are very difficult to wet and, in these cases, the slide, before the cover-glass is put on, may be gently warmed over a small flame in order to expel most of the air. Another method of expelling air, which has been found particularly useful for the biverticillate *Penicillia*, is to tease out the specimen in alcohol, then displace this with two or three successive large drops of lacto-phenol, taking up most of the fluid on filter paper before each subsequent addition. It is usually impossible to make good slides by using mounting fluid in such quantity that it just fills the space below the cover-glass (this is the practice when Canada balsam is used as mounting medium). Generally an appreciable amount oozes out, especially if the cover-glass is gently pressed down with a pair of forceps, and must be absorbed on bits of filter paper carefully applied to the edge. In the case of all slides which are intended to form a permanent record, and all temporary ones which are to be examined under an oil-immersion lens, the edge of the cover-glass must be sealed with a suitable cement, in order to prevent movement. Brown shellac cement answers the purpose admirably if properly applied. It should be thinned with alcohol until it runs smoothly but not too readily from a brush. Using a turntable, a first very thin coat should be applied and, when this is quite dry, a second thicker coat is added. Any attempt to put on too much in the first coat will probably spoil the slide, through drops of cement working their way under the cover-glass. All slides should be adequately labelled as

soon as made, giving the name of the species, the stain (if any) and the date of preparation.

IDENTIFICATION OF SPECIES

Methods of obtaining pure cultures and general methods of examination having been discussed, it now remains to describe the routine to be followed when it is desired to identify an unfamiliar species.

The first necessity is to prepare a full and accurate description of the mould, as grown on standard media. It is assumed that some idea of its general behaviour on one or two media has already been gained during isolation and purification. If the species grows satisfactorily on Czapek agar this should now be used, but if the Czapek medium gives poor and stunted growth, or if sporangia structures are lacking or tardily produced, some other medium must be selected. Several cultures should be made in Petri dishes. One or two of the dishes may be planted with single colonies for measurement of growth rate, the rest with several colonies spaced about an inch apart. It is necessary to make a number of cultures because some of them will be examined, probably with the dishes uncovered, before maturity, and are likely to develop infections on continued incubation. Cultures should be examined at frequent intervals and the following details recorded :

1. Rate of growth ; described as slow, very slow, moderate, rapid, etc.
2. Colony colour and colour changes ; whether uniform or in zones or patchy. Evanescent colours which are often to be observed at the edges of growing colonies should be recorded.
3. Colour and colour changes of the reverse of the colony.
4. Colour changes in the medium ; whether confined to the area covered by the colony or diffusing.
5. Texture of surface ; whether loose or compact ; plane, wrinkled or buckled ; velvety, matted, floccose, hairy, ropy, gelatinous, leathery, etc.
6. Odour, if any.
7. Character of drops of transpired fluid often found on aerial growth.
8. Character of the submerged hyphæ ; colour, presence

or absence of septation, approximate diameter, characters of special structures if any present.

9. The stage at which fruiting structures develop.

10. The character and disposition of the mature fruiting organs ; whether sporangia, perithecia, pycnidia, stromata, sporodochia, coremia or detached conidiophores ; whether borne in the substratum, on the surface or on the aerial growth. The presence of more than one type of sporing structure should be particularly noted.

11. Colour, size and shape of mature fruiting organs or fruit-bodies.

12. Details of structure of the fruiting organs, including measurements of essential parts and disposition of the spores thereon.

13. Full details of spores ; colour, shape, septation, surface markings, size (including both average and extreme measurements).

Data numbered 1-7 are obtained by examination of cultures with the naked eye or with a hand lens, 8-11 by the use of a low or moderate power of the microscope, observations being made on the living cultures. Numbers 12 and 13 necessitate the preparation of slides and the use of the highest powers of the microscope.

The information recorded under 8 and 10 should be sufficient to place the species in its correct class and order. For example, if the vegetative hyphæ are comparatively coarse and without septa, and sporangia are observed, the fungus must be placed in the Zygomycetes, and in the order Mucorales. Consideration of the rest of the data will lead to the family and then the genus. The determination of the actual species is, except in a few genera, a matter of some difficulty. If the genus has been monographed it is usually a question of patient and careful observation, along the lines indicated by the authority, then repeated consideration of the data until the unknown fits into its proper place. In absence of an authoritative treatment of the genus the usual procedure, and the quickest in the long run, is to consult Saccardo's *Sylloge*, look up all the recorded species in the genus and follow up references to any which seem to be near the one to be identified. Unfortunately, many species have been inadequately described and

there are even numerous genera which are ill-defined. In some cases the accepted conception of a genus is a matter of tradition rather than adequate diagnosis, and it is difficult to find any published data sufficiently exact for recognition. However, the genera which are of commonest occurrence and greatest importance are just the ones which have been most studied and concerning which there is an extensive literature.

One thing the student should guard against. It is doing a great disservice to other mycologists to assume too hastily that an unrecognized fungus is a new species and to publish a description under a new name. The literature is cumbered with a mass of generic and specific names which are nothing more than synonyms of well-known forms, and which ought never to have been bestowed.

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- This, as well as other books on photomicrography, describes fully the correct use of the microscope, since the main essential in photomicrography is to obtain the best possible microscopic image.
- BECK, C. (1938). The Microscope. 2nd Ed. London: R. & J. Beck, Ltd.
- The first edition was in two volumes, Elementary and Advanced. For the new one-volume edition much elementary descriptive matter has been omitted. It discusses the practical applications

of modern theories of microscopical vision and is a stimulating book for those who wish to get the very best out of their instruments.

COLES, A. C. (1921). *Critical Microscopy*. London : J. & A. Churchill.

This is a somewhat unconventional book which describes a very convenient lay-out for microscopic work and treats of some of the finer points of microscopy.

HIND, H. L., and RANGLES, W. B. (1927). *Handbook of Photomicrography*. 2nd Ed. London : George Routledge & Sons, Ltd.

The remarks on "Barnard and Welch" apply also to this book. It contains some beautiful examples of photomicrographic practice, with full details of the apparatus and methods used.

CHAPTER X

PHYSIOLOGY OF MOULD FUNGI

The growth of fungi, like that of all other living things, is profoundly influenced by environment. Variations in external conditions may not only affect rate of growth but, in many cases, can bring about differences in type of growth. To make a very broad generalization, fungi resemble the higher plants in that they produce vegetative growth, that is mycelium, when food is abundant and conditions are favourable for easy assimilation, and produce fruit-bodies in response to conditions which tend to check rapid growth. In addition, unfavourable environment may result in dwarf forms being produced or in the appearance of structural abnormalities. In some cases the difference between normal and abnormal growths of a single species is as great as that between different species or even different genera.

The various topics treated below are only discussed in broad outline and there has been no attempt to take account of all the great mass of published papers relating to the subject.

Food Requirements. The chemical elements (apart from water and oxygen) which are known to be necessary for growth of fungi are carbon, nitrogen, phosphorus, potassium, sulphur and magnesium. In addition, many require, or grow very poorly in absence of, iron. Opinions vary as to the necessity of a few other elements but, if any are required, the amounts are so minute as to make special provision of such unnecessary in culture media. Most of the common moulds can utilize inorganic sources of all the elements except carbon, but a few are unable to use inorganic nitrogen. The great variety of natural substrata which are commonly infected by numerous species of moulds indicates that these organisms can tolerate

wide variations in concentrations of the essential elements and can utilize both carbon and nitrogen combined in very diverse forms.

The actual quantity of food material required to support mould growth is very small. Fungi are frequently to be found growing on polished furniture woods, or even on old iron, where the total amount of available food must be exceedingly minute ; scientific workers in the Tropics have often recorded that microscope objectives have been rendered unusable by mould growth adhering firmly to the glass ; textiles made of cellulose—cotton, linen and artificial silks—may be extensively “mildewed” without the fabric showing the least sign of weakening or tendering, the only available food material being apparently the small amounts of substances other than cellulose which are contained in all such fabrics ; solutions of many inorganic salts, on standing, often develop slimy growths of mould mycelium and it is common experience that many species can grow and produce spores on plain agar made up with tap-water, a medium which, since the agar is not utilized, is extremely poor in nutrient material.

Respiration. All fungi require oxygen for growth. Many of the yeasts develop characteristically when completely submerged in liquid, where the amount of available oxygen is small, and a few other fungi produce atypical structures, resembling the yeasts, when grown under similar conditions, but no fungus is truly anærobic. The spores of a great many filamentous species will germinate when immersed in liquid media but grow very slowly until some of the hyphæ reach the surface, after which they spread rapidly and normally until the surface is covered.

Normal metabolism results in the breakdown of some of the organic food material to CO_2 and, unless this is continually removed and replaced by a fresh supply of air, growth ceases, whether on account of the diminution in oxygen tension alone or by actual poisoning is not known. In the laboratory, vessels in which fungi are grown are usually plugged with cotton-wool, and it might be supposed that this would restrict aeration. Moulds are commonly grown on liquid media in test-tubes 6" long, the depth of liquid being about 1", and it would be reasonable to assume that, with the tube in a vertical position, CO_2

would accumulate above the surface of the liquid. In a culture flask, with a much greater active surface relative to the plugged opening, conditions should be much worse, yet analyses of air drawn from flasks containing actively growing moulds invariably show that there must be a very rapid removal of CO_2 and intake of fresh air.

It is readily shown, by passing measured volumes of air through a culture vessel, or through a number of vessels in series, that lack of air influences rate of growth and, in many cases, alters the type of growth. One common effect of very restricted aeration is to suppress normal colour production and a very striking instance of this is mentioned by Thom in *The Penicillia* (1930, p. 83). He states that when Roquefort cheese is cut the fresh surface is often colourless but turns green very rapidly on exposure to air.

Reaction of Medium. Most of the commonly occurring moulds will tolerate a wide variation in hydrogen-ion concentration provided other conditions are favourable, but, as might be expected, different species respond in various ways to changes in reaction. In general, a slightly acid medium is favourable to spore germination and rapid growth of the young colony, just as a slight alkalinity is preferred by the majority of bacteria. Once growth is established the reaction of the medium usually changes owing to the accumulation of products of metabolism. Many species form fairly large quantities of organic acids, oxalic, citric and gluconic acids being very commonly found and many others more rarely. In absence of metabolic products of a strongly acid nature, alteration in reaction of the culture medium depends largely on the types of inorganic salts present. For example, the utilization of nitrogen from sodium nitrate tends to liberate base and increases the $p\text{H}$, from ammonium sulphate to liberate acid and decrease the $p\text{H}$, whilst the presence of appreciable amounts of buffer salts tends, of course, to stabilize the reaction. It is not unusual for appreciable quantities of organic acids to be produced during the early stages of growth and then to be utilized by the mould as other sources of carbon become exhausted. In such cases the $p\text{H}$ of the medium will first decrease and then gradually increase as growth proceeds. Sometimes the appearance of the mould itself indicates changes



FIG. 125.—*Phycomyces nitens*—culture in Roux bottle, illuminated from one shoulder only during period of growth, showing well-marked phototropism. $\times 0.5$.

in reaction of the medium. In many of the green species of *Aspergillus* and *Penicillium* the green colour of the young colony is very stable, often persisting for several months, on a heavily buffered, acid medium such as wort, whereas on Czapek's medium, which tends gradually to become alkaline unless strong acids are synthesized, the initial green slowly changes to brown or grey shades. Many mould pigments, produced either in the mycelium or in the medium, act as indicators, showing very distinct colour changes according to alterations in reaction.

Influence of Light. It is impossible to generalize concerning the effect of light on the growth of fungi for, whilst many common species seem to grow equally well and characteristically in light or in darkness, others are definitely stimulated or affected by light. For example, a culture of *Rhizopus nigricans*, growing in a Petri dish, was clamped on the microscope stage and the edge of the colony was observed with a 2" objective and a low-power eyepiece fitted with a micrometer scale (magnification approximately 15 ×). In diffused daylight the extension of the radiating hyphæ could actually be seen and, after an hour and a half the edge of the colony had advanced almost half the diameter of the field. When the whole apparatus was placed in the dark the advance was less than half that observed in the light, showing that, in this species, there was a very definite stimulation of vegetative growth by comparatively weak light. Some fungi are positively phototropic; that is, they grow towards the light or their fruit-bodies turn in the direction of maximum illumination. The sporangiophores of *Phycomyces nitens* are short when a culture is fully exposed to light but attain a length of 20–30 cm. if the mould is grown on a layer of medium placed at the bottom of a tall vessel which allows light to enter only from the top. Fig. 125 shows a very characteristic growth of *Ph. nitens* in a Roux bottle which was covered with opaque material except for a patch on one shoulder, the sporangiophores being all turned towards the point of entry of the light. A number of other species exhibit similar tendencies. The sporangia of *Pilobolus* species regularly incline in the direction of strongest illumination; the stipe of the wood-destroying fungus, *Lentinus lepideus*, grows towards the light and the cap develops only when the intensity of the light exceeds a certain minimum;

the necks of the perithecia in some of the Pyrenomycetes are regularly orientated towards the light ; and it is probable that, in all such cases, phototropism represents an adaptation for the better dispersal of spores.

The effect of light on some species is to stimulate the production of fruit-bodies, exposure to sunlight or ultra-violet rays being often a useful method of inducing sporing in cultures which have become purely mycelial. Pigment production is also quite frequently initiated or enhanced by the influence of strong light.

Temperature Relationships. Fungi show great differences in their response to temperature changes and in their powers of resistance to heat and cold. Application of heat is, of course, the usual method of sterilizing culture media, vessels and tools and of killing unwanted cultures, there being few moulds which can withstand the action of steam or boiling water for any appreciable time. Thermal death points, however, cover a wide range, some strains of *Penicillium brevicompactum*, for example, being killed by prolonged exposure to a temperature of 33° C., whilst *Byssosclamyces fulva*, a fungus responsible for much trouble in the canning industry, can survive the normal sterilization process in which, for a short time, the temperature exceeds 90° (Olliver and Smith, 1933). On the whole, spores are more resistant than mycelium and both are less affected by dry than by moist heat. In the same way the spores of many species are killed by freezing in presence of water but when dry have their germinative powers unimpaired by prolonged cooling in liquid air.

The limiting temperatures which will admit of growth, as distinct from mere retention of vitality, are less extreme. It has frequently been recorded that *Cladosporium herbarum* will grow on meat products in cold storage at a temperature of - 6° C., and this probably represents the lower limit. At the other end of the scale, *Aspergillus fumigatus* is thermogenic as well as thermophilic and is often found flourishing about 50° C., a temperature at which nearly all other fungi are inhibited and many of them killed. Quite a number of common moulds, including many *Mucors*, *Aspergilli* and *Penicillia*, grow well at 37° C., but many others are inhibited.

Each species is found to grow best at round about some

particular temperature, known as the optimum temperature for that species. On the whole, species of *Penicillium* have optima between 20° and 25° C., whilst the nearly related *Aspergilli* grow best at about 30°. This is a very rough generalization, with many exceptions, but is often of value in industrial work, where 90 per cent of the fungi encountered belong in these two genera. One particular section of the *Aspergilli*, the *A. glaucus* group, deserves special mention, as most of the strains have two optimum temperatures. Conidia and purely vegetative growth are produced most freely at somewhat low temperatures and, therefore, temperature optima, as determined from growth rate, are low, for some strains as low as 10°. Perithecia, on the other hand, are formed most readily and abundantly at higher temperatures, some species being almost entirely perithecial above 30°, and there is thus a second optimum relating to the perfect form of the fungus. Although the majority of the *Penicillia* have optima which are comparatively low, there are a number of species, mostly belonging in one group, the *Biverticillata-Symmetrica*, which show maximum growth at temperatures near to 30° and which flourish up to at least 37°.

Moisture Requirements. A supply of water is absolutely essential for the growth of all fungi and, although a few species can thrive in presence of very small amounts of available moisture, it is fortunate that the great majority of moulds require what may be termed damp conditions. Dry rot appears only on timber which has not been thoroughly seasoned or which is exposed in a humid, stagnant atmosphere, and mould appears on wall-paper only when joints in the wall are faulty and the paper itself has become perceptibly damp. Many industrial products, paper, textiles, leather, and food-stuffs of various kinds, readily absorb or adsorb water from a humid atmosphere and are liable to attack by but a very limited number of moulds except when stored under conditions where this intake of water is excessive.

It is important to realize that the total amount of water contained in any particular substance does not necessarily determine its liability to fungal attack. Jam, for instance, contains a high percentage of water but, if properly compounded, does not become mouldy because the high concentration of soluble material, chiefly sugar, renders the water

unavailable to fungi, whereas a material consisting chiefly of cellulose, such as cotton or paper, is liable to attack if the moisture content exceeds about 8 per cent. Glycerine is often considered to have antiseptic properties but, at low concentration, it is an excellent food for many fungi and it inhibits growth only in concentrated solution, when the effect is chiefly or entirely due to its desiccating action and not to any specific toxicity. Of all the fungi which can tolerate high osmotic concentrations of dissolved substances easily the most important are members of the *Aspergillus glaucus* series. These are frequently encountered on all types of material which contain only very small amounts of excess moisture. They are commonly found, as pure cultures, on jams and similar products which contain rather less than the normal percentage of sugar.

The question of moisture requirements is further discussed in Chapter XII.

Poisons. The Fungi, like other classes of living things, are affected adversely by the presence of certain substances in their food supply. Our knowledge of what might be termed poisons has been gained chiefly through the demands of various industries for efficient antiseptics to combat the ravages of fungi amongst their raw materials and products. This topic is discussed further in the chapter on "Control of Fungi" (Chapter XII) and will not be elaborated here.

It is an interesting fact that the use of certain poisonous substances, in very small doses, as general tonics for the human system is paralleled by the use of small quantities of antiseptics for stimulation of fungal growth. Zinc salts are probably the best known of such substances and are regularly incorporated in certain well-known culture media, but many other substances show the same reversal of effect on particular species or groups of species.

Influence of Other Fungi. If several species of fungi are growing together on the same substratum they may affect each other's growth in various ways. In the simplest case there is competition for the available food material and the ultimate result is that some species are prevented from spreading, or starved out of existence, whilst others flourish. It is impossible to predict, from knowledge of the individual species, which will be the survivors in a mixed culture, since rapid growth in pure

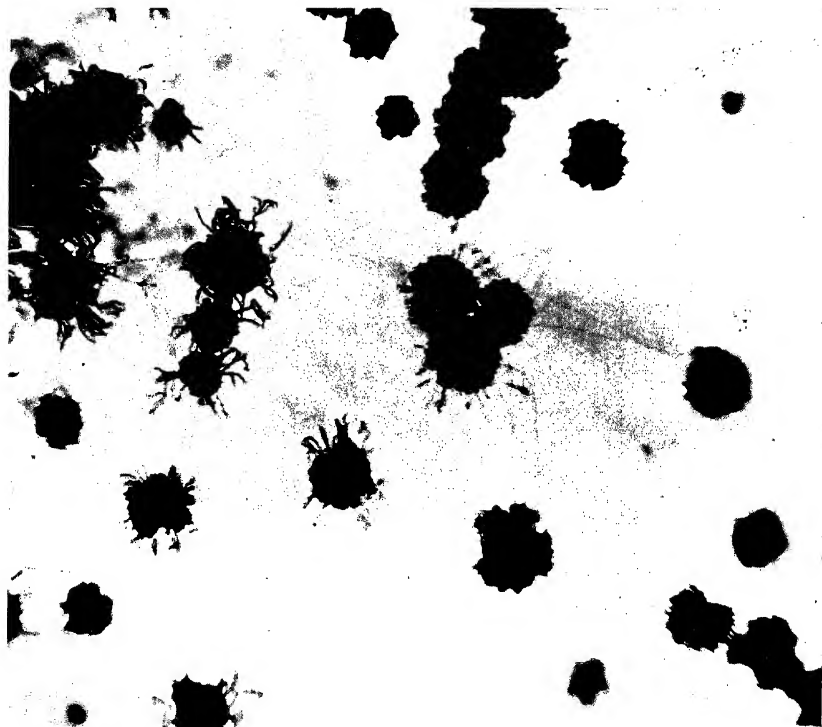


FIG. 126.—*Penicillium* disease of *Aspergillus niger*—infected heads
as seen in Petri dish. $\times 25$.

culture does not necessarily mean ability to acquire food in face of competition. In many cases of mixed infections it is found that some of the moulds grow abnormally, producing freak structures or becoming dwarfed, and it is therefore very unsafe to attempt to base identifications on examination of such material. A very common state of affairs, when a suitable substrate is exposed to chance infection, is for there to occur a well-marked progressive change in the fungal population, probably correlated with the different enzymatic activities of the various types. One group breaks down complex organic material to provide suitable food for another group, whilst a third group will utilize the metabolic products of the second. Many fungi, for example, are unable to attack cellulose but flourish luxuriantly on the products of its partial decomposition. Amongst the coprophilous fungi there is usually a well-marked sequence, the primary flora of dung consisting of Mucorales, followed by Ascomycetes, then by Basidiomycetes.

True parasitism amongst the fungi is by no means uncommon. The higher fungi, particularly the large fleshy forms, are attacked by a large variety of micro-fungi and even a few of the forms commonly grown in the laboratory are parasitized, usually by nearly related species. Thus a number of Mucorales, most frequently species of *Chaetocladium* and *Piptocephalis*, attack other members of the same order, and *Penicillium rugulosum* grows on cultures of various *Aspergilli*, most commonly *A. niger*, forming masses of olive-green fruits over the heads, and eventually killing them (see Fig. 126).

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CHAPTER XI

THE MAINTENANCE OF A CULTURE COLLECTION

In most laboratories where serious work on micro-fungi is carried out it is necessary to maintain a collection of moulds. If the work in hand aims at controlling the harmful activities of fungi on some industrial product most of the preliminary work will have to be done on pure strains of moulds isolated from actual cases of damage, and every important species will have to be kept in pure culture so as to be available for numerous experiments extending, perhaps, over a period of years. Those who study the biochemical activities of fungi, with a view to isolating or manufacturing special products of metabolism, usually find it necessary to test many strains of the same or related species, to grow them all on many different media and under varying conditions and, again, a type collection is essential.

Requirements of different laboratories, as regards size and type of collection, will vary greatly, but there are a number of general methods and precautions whose consideration is applicable to all cases. The main essentials may be briefly summarized thus :

1. All cultures must be kept alive as long as required.
2. Every culture must be maintained in a state of purity.
3. As far as possible, each species or strain must be kept true to type, that is with all the characteristics it had when first introduced to the collection.

The chief essential in keeping moulds alive is transfer at sufficiently frequent intervals. Different species, even of the same genus, vary much in longevity, the spores of some common moulds remaining viable for years whilst others lose their power of germination after a few months. Some cultures

can withstand desiccation and may readily be sub-cultured even after they have dried up to a horny mass, whilst others, chiefly those which do not spore freely, must be kept on a moist medium and die off if allowed to become even approximately dry. The frequency with which sub-culturing of a collection is necessary depends very largely, therefore, on the peculiarities of the particular fungi, but it also depends to some extent on external conditions, particularly on the temperature of the place of storage. If kept in a room where the average temperature is 18–20° C. most of the common moulds may be kept alive by transferring two or three times a year, whilst yeasts and related fungi should be sub-cultured about every two months. If the place of storage can be maintained at a temperature of about 10° C., cultures will remain in good condition for a longer time and the majority of species may safely be left eight or nine months between transfers. With such a place of cold storage available it is best to grow sub-cultures in the 25° incubator until characteristic features have developed sufficiently to decide whether the transfers have been completely successful and then to remove the cultures to the 10° storage chamber, where growth continues more slowly. A few species, notably those belonging to the *Aspergillus glaucus* group, grow well at 10° C., some of them better than at 20°. Even in these cases the lower temperature is suitable for storage since its chief advantage is that drying out of the medium is retarded. When special accommodation at a low temperature cannot be provided experience will soon show how often the collection should be sub-cultured, but it is always better to err on the side of frequency, since the loss of a valuable culture is very annoying.

As regards choice of media for stock cultures it is best to aim at encouraging growth which is characteristic but not too luxuriant. For maintaining vigour in slow-growing and delicate species wort agar is ideal, but many of the more strongly growing species tend, on this medium, to produce masses of aerial mycelium at the expense of typical fruiting structures. Czapek agar is not so rich a medium as wort and is ideal for a great many species. Potato agar and prune agar are intermediate and are particularly valuable for many of the Mucorales, which completely choke up the culture tubes when grown on wort but grow very poorly on Czapek agar. Gelatine

media are, on the whole, unsuitable for stock cultures, as the gelatine is readily liquefied by very many moulds. In any collection it is advisable to keep at least two, and preferably three stock cultures of each mould as an insurance against loss by accident or contamination, and these may well be on different media, since a periodical change of diet seems to preserve vigour and character in many species.

It sometimes occurs, on account of delay in sub-culturing or even for no apparent reason, that transfers made in the usual way fail to grow. It is unwise to assume at once that the old culture is dead. A method of saving the species, even when only a few viable spores remain and when the chances are that repeated needle transfers will effect nothing, is to flood the surface of the old culture with a small quantity of sterile liquid medium and incubate. The new medium provides sufficient moisture and nutriment to bring about the germination of any spores which are still alive, after which sub-cultures may be made in the usual way. Another method is to scrape off as much as possible of the old growth in about 3 c.c. of sterile water, then flood this suspension over the surface of a suitable agar medium in a Petri dish.

The problem of keeping stock cultures in a state of purity is beset with more difficulties than is the task of merely keeping them alive. In the first place it is obvious that sub-culturing a number of species, one after the other, demands the utmost care and faultless technique. Even, however, when every care is taken, infection will sometimes occur if the work is done in a highly contaminated atmosphere. Sometimes it is unavoidable that cultures are handled in such a way that clouds of spores are discharged into the air. If this happens in a room where transfers of stock cultures have to be made, it is well, before starting to sow, to minimize the risk of infection by sterilizing the air, using one of the methods described in Chapter IX.

Even the best workers are troubled by occasional contamination of cultures and it is therefore very necessary to be able to recognize such when it does occur. Infection of a culture by a species of a different genus, say the invasion of a *Mucor* by a *Penicillium*, is easily seen on casual inspection, but infection by a closely related species of similar appearance may go unnoticed for a long time and may even result in complete loss

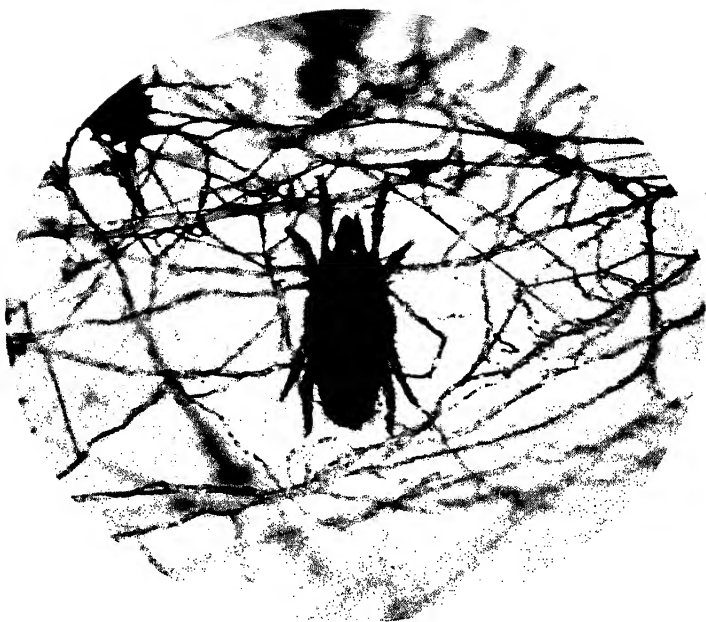


FIG. 127.—Mite (adult) in culture tube. $\times 100$.
The larvæ are somewhat similar in appearance
but have only six legs.

of the original and its replacement by the contaminant. The main essential, the importance of which cannot be over-emphasized, is that the worker in charge of the collection should keep full records of all species, noting gross appearance at various ages, rate of growth and microscopical appearance, both of the living cultures and of mounted specimens. It is true that an enthusiastic mycologist in time gets to know all his moulds as well as the keen gardener knows all the plants in his garden but, even then, records are more valuable than memory alone and should never be omitted or scamped. Periodical examination of the collection should be made and, if there is the slightest suspicion of anything wrong, the particular species should be plated out, fresh cultures being made from typical colonies.

When the culture collection includes species of several different genera it will be found that chance infection due to imperfect methods of transfer is more readily spotted if the species are stored and sub-cultured in random order. For example, number 1 in the collection may be an *Aspergillus*, 2 a *Mucor*, 3 a *Cladosporium*, 4 a *Penicillium* and so on throughout, with never more than two or three of the same genus bearing consecutive numbers. A collection of species of a single genus, particularly one in which there is little colour variation, is far more likely to carry unnoticed contaminants than is a miscellaneous collection.

Mites are a most annoying pest to have in the culture laboratory and are one of the most serious causes of contamination. A collection of fungi may remain unattacked for years and then, suddenly, scores or hundreds of cultures are found to be infected, but, unless the mycologist is constantly on the look out, and unless he knows what to look for, the depredations may go unnoticed for a long time and may result in wholesale contamination and loss of many species. Fig. 127 is a photomicrograph of a typical mite in a tube culture. The different species vary somewhat in shape and size, but, when once seen, can never be mistaken for anything else. An adult mite is usually about one-hundredth of an inch in length and is thus almost at the limit of unaided vision. When alive and in motion they are, as a matter of fact, readily seen, as small whitish specks, by anyone with normal sight but, if any cultures

are known to be infected, or there is any reason to suspect the presence of mites, *all* cultures should be carefully examined under the microscope, using a 2" objective. The evil the mites do is twofold. In the first place they eat the cultures and, if left unchecked, may even destroy them entirely. In addition, they crawl from one culture to another, with spores adhering to their hairy bodies, spreading infection wherever they go. Petri dishes are readily entered and become contaminated with amazing rapidity if once the mites appear in their vicinity. Cultures in tubes are just as readily invaded, since the mites find no difficulty in crawling through the tightest cotton-wool plugs and according to Thom, they have even been known to find their way through paraffined plugs. If the plugs are a good fit the mites shed most of the adhering spores *en route* to the cultures, the major portion of the damage being then due to their gastronomic activities, but mixing of cultures does occur and is presumably due to spores which pass unchanged through the digestive tract.

The control of mites is not easy. They are brought in with all kinds of raw materials, they may be introduced with new laboratory fittings and apparatus or they may occur in cultures sent from other workers, and hence hygienic measures sufficiently stringent to prevent their access to the culture room are difficult to realize in practice. Constant vigilance is necessary, with the prompt application of suppressive measures at the first indication of an attack. In some laboratories it is the custom to paint bands of paraffin and rubber tap-grease round the rims of the culture tubes, but such tubes are messy to handle and the treatment is of doubtful efficacy as a method of prevention, for mites have actually been observed to crawl through such a grease band, very slowly, it is true, but with a sure sense of direction.

The usual method of killing mites is to expose the infected cultures to the vapour of either carbon tetrachloride or pyridine. Either is quite effective against the actual mites, but there are two stages in the life history of these creatures, the egg and the "hypopus," which are most resistant and which cannot be killed with certainty by fumigation. Wherever mites have obtained a foothold their eggs will be found in large numbers. They are mostly oval and pale coloured, but

are frequently not easy to recognize amongst the fungus debris which is always present in an infected culture. They hatch out within a few days at the most and a second fumigation, three or four days after the first, will kill off all the young mites and should leave the cultures free from eggs. Hypopi are an encysted stage between the young and the adult, occurring only in some species and under conditions which are not completely known. Unfortunately the metamorphosis to the adult stage does not take place after any definite time and the hypopi themselves are very difficult to kill. If they are observed it is necessary to wait, with frequent examination of the cultures, until the adult form emerges before carrying out the second fumigation. The best method of conducting the fumigation is to place all the infected cultures in any suitable receptacle which can be tightly closed—a bell-glass on a well-fitting base is quite suitable or a spare incubator may be used if available—place along with them one or two shallow dishes of the fumigant and leave for twenty-four hours. It is surprising how quickly the vapour, under these conditions, will penetrate inside tightly plugged tubes. It is advisable to treat, at the same time, the boxes or cupboards where the cultures are normally stored, in order to kill any mites which have lodged in the joints of the woodwork. Opinions vary as to the effect of the vapour treatment on the moulds themselves, some maintaining that carbon tetrachloride is unsafe, others that it is to be preferred to pyridine. In the experience of the writer either may be used without ill effect and it then becomes chiefly a question as to which is less objectionable to the individual worker. One curious difference is that mites exposed to carbon tetrachloride curl up when they die whilst pyridine leaves them extended as in the photograph, Fig. 127. One precaution should always be taken—fresh cultures should be made directly it is ensured that all the mites are dead.

Finally it remains to be considered how far it is possible to keep moulds, through a series of sub-cultures, true to type. Some moulds vary little or not at all, successive transfers coming up in every way, as far as can be seen, identical with the original. Unfortunately, on the other hand, a few fungi seem to be decidedly unstable in artificial culture and defy all efforts to maintain their distinguishing features. Between these two

extremes are a large number of species which can be kept in good condition only if special precautions are taken. Amongst these the *Fusaria* stand out as a somewhat special group since it is known that the major cause of atypical cultures lies in the method of transfer when making sub-cultures. This question has already been dealt with in the description of *Fusarium* (Chapter VI) and needs no further mention. Apart from the *Fusaria*, however, a number of other moulds tend, after repeated sub-culturing, to produce sterile mycelium at the expense of fruiting structures. For example, species of *Chaetomium* tend to lose the power of producing perithecia; many of the Dematiaceæ, in particular species of *Alternaria*, *Stemphylium* and their near relations, often produce masses of floccose, whitish mycelium over a thin basal felt of the typical dark-coloured hyphæ, and spores are formed very tardily and sparingly or not at all; strains belonging to the *Penicillium luteum* group frequently become purely mycelial, lacking both perithecia and conidia; even some of the *Aspergilli*, normally amongst the least troublesome of all fungi in this respect, occasionally deteriorate and become almost sterile. In all these cases it is not known that the type of material transferred, when sub-culturing, has any effect on the resultant growth, as with the *Fusaria*. Experiments with certain fungi have shown that the type of culture is largely determined by the amount of nutriment in the medium, the findings being in accordance with the generalization at the beginning of this chapter. For example Coons (1916), in an investigation of cultural variations in *Plenodomus fuscomaculans*, found that pycnidia were produced only when the source of carbon was present in less than a definite limiting concentration. It is well known that strong light tends to induce sporulation and it is always advisable to try the effect of short exposures to bright sunlight on any cultures which are showing undue development of sterile aerial mycelium. Many of the pathogenic *Helminthosporia* do not spore at all on artificial media under ordinary conditions, but Weston (1933) has shown that *H. avenæ* can be induced to give normal conidiophores if cultures are submitted to one or two short irradiations from a quartz mercury vapour lamp. Doubtless there are other factors involved, besides food and light, and much more work will have to be done before we

can generalize on the subject of cultural degeneration and its prevention. At present, the worker who is faced with the problem of restoring sterile cultures can only experiment.

Another type of deterioration is, fortunately, not common but, when it does occur, is more serious in that it usually results in total loss of the species. Growth in successive transfers remains fairly typical but becomes less and less vigorous until the vitality of the fungus seems to dwindle entirely away. If the preservation of such a strain is desirable it is necessary to carry out a fairly extensive cultural investigation, trying the effect of different sources of carbon and nitrogen, of the addition of minute amounts of metals other than the usual combination (zinc, for example, seems to be essential in a few cases and acts as a stimulant in others), of adjusting the medium to different reactions, of growing the fungus at various temperatures and so on, until a set of conditions can be formulated which will ensure vigorous growth.

Besides these kinds of variation, more or less responsive to environmental conditions, there is another important cause of change inherent in the fungus itself. Mutation is not uncommon in most genera of fungi and provides a fascinating subject of study. It is commonly evidenced by what is known as "sectoring," a more or less circular colony of the mould showing one or more fairly clean-cut sectors, which vary from the rest of the colony in such characters as colour, texture and degree of sporulation. Sectoring may often be induced by abnormal conditions, such as a temperature near the maximum for growth, or the presence of small amounts of toxic substances. The new type of growth, in these cases, may either be stable or may revert back to the original on restoring to normal conditions. Some species regularly give rise to true mutants when grown in the normal way and, if the new strain happens to be of more vigorous habit than the parent, the latter may be entirely swamped unless the change is noticed in good time and the two forms separated.

The phenomenon of heterothallism, as a case of apparent degeneration of cultures, has already been discussed in Chapter I.

A final form of variation from type, which the biochemist knows only too well, concerns, not the appearance or structure of the fungus, but alteration in chemical activity or loss of the

power to produce some particular metabolic product. Two examples, from amongst the many recorded in the literature, may be cited to illustrate this type of change. Wehmer, in 1918, described a new species of *Aspergillus* which gave good yields of fumaric acid when grown on glucose media and which was therefore named *A. fumaricus*. In a later paper, 1928, he reported that his fungus no longer produced fumaric acid but gave gluconic acid instead. Birkinshaw and Raistrick (1931) describe the production, by four strains of *Penicillium* related to *P. spinulosum* Thom, of a substituted toluquinone which gave to the culture medium an intense purple colour. All the strains, when freshly isolated, gave appreciable amounts of the specific product, but all gave gradually decreasing yields, and, after repeated sub-culturing, refused to produce the least trace of the substance. This kind of variation gives rise to serious difficulties when an attempt is made to utilize the metabolic activities of fungi for the large-scale production of substances of industrial importance. Before any such process can become a commercial proposition it is necessary to carry out much preliminary work in order to ensure that any tendency to loss of activity can be completely controlled.

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CHAPTER XII

THE CONTROL OF MOULD GROWTH

The growth of moulds in undesirable places is the cause of almost incalculable loss in industry and of less serious, but still extensive, damage in many households. It is therefore desirable that the available means for combating their ravages should be as efficient as possible and generally known.

Much may be done to minimize trouble from moulds in the factory by strict attention to hygiene. Damp and dirty walls, corners of floors and ceilings which are not easily cleaned, or waste organic material left lying about are all liable to develop patches of mould and act as reservoirs of infection, the spores being carried to all parts of the factory by air currents. Leaky steam-pipes or water condensed from process steam on ceilings and parts of machinery may cause drips on to valuable material, creating conditions eminently suitable for the growth of fungi. Suitable precautions to prevent infection from such sources as these are obvious. In some cases, where the neighbourhood is excessively dusty or where there is a known local source of airborne infection, it may be advisable to purify the air entering the building.

Even with ideal factory conditions, however, it is seldom possible to prevent entirely the access of mould spores to factory products. Latent infection may be carried in raw materials, complete sterility of the air in a work-room is almost impossible to achieve owing to air currents created by movements of materials and workpeople and the latter may introduce spores in large numbers on their clothing. Therefore, even if damage by moulds within the factory be completely prevented, there remains the problem of ensuring that products remain in good condition after they leave the factory and until they eventually

reach the consumer. There are three main lines of attack on this problem :

1. To ensure continuous sterility by preventing the access of mould spores to an already sterile or sterilized product.
2. To arrange that an industrial product is kept, or keeps itself, in such physical condition that growth of moulds is limited or prevented entirely.
3. To limit mould growth by means of toxic substances, usually known as antiseptics.

The first method is of very limited application but is usually, and should be always, effective in cases where it can be carried out. The best-known application of the method is, of course, the food-canning industry. In the canning of meat, fish and certain vegetables the main object of sterilization is to destroy bacteria but, since moulds are less resistant to heat than are the majority of bacteria, any cooking operation which will destroy the latter will also automatically kill any moulds present. In the fruit-canning industry, however, high temperatures and long cooking have to be avoided, as they detract from the appearance of the products. Fortunately the acidity of most fruits is of great assistance in suppressing bacterial growth and processing can therefore be conducted at temperatures below 100° C. As already stated in Chapter IV one fungus, *Byssoschlamys fulva*, has caused considerable trouble in the fruit canneries owing to its ability to withstand a temperature only a little below the maximum attained in processing, necessitating greater care being taken to ensure that all parts of the contents of the cans reach the safe temperature (see Olliver and Rendle, 1934).

One branch of the food industry, the packing of fruit for export, applies the principle of isolation in a special way. The moulds causing rot of fruits are specialized parasites and thus, if the access of spores of *Penicillium digitatum* and *P. italicum* to citrus fruits can be prevented, the main cause of spoilage is removed. A good deal of work has been done on the sterilization of citrus fruits by means of antiseptic washes, chiefly solutions of borax or boric acid. A fair measure of control is thus obtained, but a small proportion of fruits still remain infected. If packed without further precautions infection may spread and result in serious loss, but, if the fruits are wrapped

in waxed or chemically treated papers, any infected fruits may be extensively rotted and covered with a thick growth of mould without infecting any other fruits in their vicinity. A good account of the control of citrus moulds is given by Fawcett (1936). Wrapping of apples has similarly diminished to a remarkable extent losses due to *Penicillium expansum* rot. It is not unusual to find, in a case of wrapped apples, one or two fruits which are nothing but a mass of brown rottenness, whilst the rest are quite unaffected.

A method of partial sterilization which has received considerable attention during recent years utilizes the lethal properties of ultra-violet radiation. It has been found that both mould spores and bacteria are rapidly killed by exposure to a source of light rich in ultra-violet rays, although it is doubtful how far the effect is due to the direct action of the rays and how far to the action of the ionized oxygen produced in the neighbourhood of the source of light. James (1936), for example, claims that direct irradiation is unnecessary and that air circulation during the treatment is beneficial, indicating that it is ionized oxygen which is the potent factor. In any case the effect is exerted only on the surface of the irradiated material, since neither ultra-violet light nor ionized oxygen can penetrate far into most kinds of matter. This is of little consequence for such materials as bakery products, which are usually sterile as they leave the oven and become infected only on the surface during cooling. Such goods, after irradiation and wrapping in irradiated paper, are virtually sterile throughout and remain in good condition until they reach the consumer. The chief applications of the method at present are to various food-stuffs and tobacco, its use for many other materials which undoubtedly could be sterilized in this way being limited by questions of cost and difficulties of sterile packing.

In attempting to limit mould growth by physico-chemical means the most important factor which must be controlled is the amount of available moisture. Spores of fungi cannot germinate without moisture and, therefore, if the amount of water in any material can be kept below a certain critical point, fungoid growth will be entirely prevented. This critical point varies considerably for different materials and must be determined experimentally in each individual case. The reason for

such variation is that the absolute amount of water in any material seldom corresponds with what may be termed the available amount, the latter depending on the chemical constitution and physical state of the particular substance. For example, the "safe" limit of moisture in wool is much higher than the amount allowable in cotton, the difference being accounted for by their very different attractions for water. If dry samples of pure wool and pure cotton are exposed to the same atmosphere the wool will take up approximately twice as much moisture as the cotton and, leaving out of account differences due to chemical composition, the two samples will be approximately equally liable to mildew, or, if the respective percentages of moisture are below the respective safe limits, will be equally resistant.

The method of obtaining the necessary data for control of mould growth by this method is, first, to determine the relationship between atmospheric relative humidity and moisture content of the particular material over any desired range of temperature, then to expose samples infected with mould spores to atmospheres of predetermined humidities. In this way is found the lowest relative humidity, and hence the lowest percentage of moisture in the material, at which growth of moulds commences. The infection may be that naturally occurring on any particular raw material, or, preferably, the test may be made with single organisms which are known to cause damage in practice. A simple method of conducting such tests in the laboratory is to suspend small samples over solutions of calcium chloride of known strength, contained in stoppered bottles or desiccators. A series of such containers can be accommodated in an incubator in order to obtain accurate control of temperature. The table on the opposite page will be found useful by those wishing to undertake tests of this kind.

Laboratory tests give useful and necessary information but, in attempting to apply the results in practice, complications may arise, chiefly due to temperature variations. If any hygroscopic substance is freely exposed to the air the amount of moisture in the substance, when equilibrium has been reached, is a determinable function of the relative humidity of the air, but a great many industrial products are transported, stored

Grams CaCl ₂ in 100 g. H ₂ O.	Density g./ml. at 15° C.	0° C.	Relative Humidity, per cent.			
			10° C.	20° C.	25° C.	30° C.
15	1.115	92.8	91.0	89.7	89.5	(90.0)
20	1.150	87.7	87.2	87.5	86.0	(85.6)
30	1.213	76.8	77.8	77.4	76.2	(76.3)
40	1.272	66.7	67.4	66.3	65.9	66.1
50	1.325	56.9	55.6	54.6	55.3	56.3
60	1.374	<u>43.8</u>	44.8	43.9	45.2	46.7
65	1.396	”	<u>40.0</u>	”	”	”
70	”	”	”	<u>34.1</u>	35.7	38.0
80	”	”	”	”	29.9	30.9

Figures underlined indicate the presence of solid salt.

Figures in brackets are extrapolations.

and marketed in packages which are airtight or through which diffusion of air is extremely slow. The great majority of solid substances tend to lose moisture as the temperature rises, whereas the capacity of air for holding moisture is enhanced with increasing temperature. Two questions then arise, for the answering of which we have far from adequate data at the present time. How does the moisture distribution inside an airtight package, which contains solid material together with a comparatively small weight of air, vary as the temperature is changed? Also, can spores of moulds acquire the moisture necessary for germination from a humid atmosphere, even though the substrate with which the air is in contact is comparatively dry? An interesting paper by Galloway (1934) attempts to answer the second of these questions. He made use of the fact that cellulosic materials, exposed to an atmosphere of definite humidity, contain different percentages of moisture according as they are being dried from a wet state or moistened from the dry state. He was thus able to use substrata which contained different percentages of water when exposed to the same atmosphere, but were otherwise identical, and also materials, otherwise identical, which contained the same percentage of moisture when exposed to atmospheres of different humidities. The experiments showed that atmospheric moisture is more effective than moisture in the substrata

for bringing about the germination of mould spores. This fact may possibly supply the explanation of a number of mysterious cases of damage by moulds, when the spoiled material has been apparently too dry to permit growth, but it is impossible to generalize until we have an answer to the first of the above questions. There is a promising field for research along these lines.

Another factor which tends to lower the permissible limit of moisture in stored products is the possibility of local variations of temperature within the package. If one side of an airtight container be exposed to a source of heat, such as direct sunlight, a ship's boilers, or heating pipes in a warehouse, whilst the opposite side is kept comparatively cool, it is reasonable to expect that migration of moisture will occur, and the extra water acquired by the cool portions of the package may be sufficient to cause the germination of dormant spores. Against the possibility of damage arising in this way a manufacturer has no certain means of protection. The utmost he can do is to allow as large a safety margin as possible in the total moisture content of his product and to urge merchants to take suitable precautions during transport and storage of the same.

Another method of preventing mould growth, one, however, which can be used only in special cases, is to limit the amount of *available* moisture in moist materials by arranging that there is a high concentration of soluble matter, thus ensuring that the water present is in the form of a solution of very high osmotic pressure. The best-known example of this method is the manufacture of fruit preserves. It has been found from experience that the concentration of sugar in the finished article must be close to 65 per cent in order to ensure sterility. With amounts of sugar only slightly less than this a few cosmopolitan species of moulds, notably members of the *Aspergillus glaucus* series and *A. candidus*, can grow quite well. Pickling in brine is another process relying for its efficacy on the same phenomenon.

The control of mould growth by the use of antiseptic substances has been practised, or at least attempted, for some considerable time. It is only in recent years, however, that organized research on the subject has put the practice on a scientific basis, explained many failures of the past, and made

available new substances of high antiseptic value and general applicability.

It should be stated at the outset that antiseptics are not necessarily fungicides. Fungus spores are often very resistant to toxic substances and most substances which will kill them are unavailable for use in industrial products because they are corrosive, give off objectionable vapours or are highly poisonous to human beings. Antiseptics merely inhibit growth and their action is limited by a number of factors, such as the availability of nutriment present along with the antiseptic, amount of available moisture, concentration of antiseptic substance and, in some cases, time. In certain cases, such as wood preservation, it is possible to use powerful antiseptics in high concentration, and effect complete protection, but often there are technical objections to the use of mould preventatives in really efficacious amounts, as well as questions of cost, and the most that can be achieved is to prevent mould growth under more or less normal conditions of use and storage. The concentration of antiseptic required to prevent growth usually increases rapidly as the humidity of the atmosphere increases, and there are a number of antiseptics which are effective at normal humidities but which become entirely useless when the relative humidity exceeds 95 per cent. There are many antiseptics which, at very low concentrations, actually stimulate the growth of fungi. With somewhat higher concentrations germination of spores is delayed and, with still larger amounts, completely inhibited. A study by Morris (1927) of a large number of antiseptics showed some interesting differences in behaviour when these were incorporated in substrata otherwise favourable to mould growth. There were naturally large differences in the amounts of the various antiseptics required for a definite retardation of growth but, apart from this, it was found that the curves relating concentration of antiseptic to degree of inhibition were of different types. With some substances the effect was strictly, or almost strictly, proportional to concentration, whilst with others there seemed to be a maximum effect, short of complete inhibition, which could not be exceeded by the use of higher concentration of the antiseptic.

An important point which is often overlooked is what may

be termed the specificity of antiseptics. Different species of fungi react differently to any particular antiseptic. A substance may give adequate protection so long as the potential infection is limited to certain species and may fail completely if spores of another species are introduced. For example, salicylic acid is a very efficient preventive of the growth of most of the Dematiaceæ and of many *Penicillia* but can actually be utilized as a source of carbon by *Aspergillus niger*. Zinc chloride is a popular and normally efficient antiseptic but, in concentrations generally used, has no effect on *Aspergillus terreus*. Morris found that salts of thallium are of high efficiency with most of the species used in his tests but very much less effective with *Cladosporium herbarum* and *Alternaria tenuis*. Hexamine, on the other hand, has comparatively low antiseptic value with most fungi but is particularly effective against the few moulds which can grow in presence of thallium. It is advisable, therefore, when considering the question of an antiseptic for protecting any particular product, to find first of all what are the usual species of moulds which infect the product, and then test out various antiseptics with these species.

The actual testing of antiseptics, so as to give results of value, is by no means a simple matter. A usual method of obtaining a preliminary valuation is to grow a number of species of moulds, in the laboratory, on media containing varying percentages of the antiseptic and to measure growth rates. The basal medium used may be one of the usual agar culture media or, better, a medium containing the particular material to be protected as sole source of nutriment, the material being used either in the form of a stiff paste or as a fine suspension in a plain agar gel. To begin with a number of media are prepared containing say 0.01, 0.05, 0.1, 0.5, and 1.0 per cent of the antiseptic respectively. A series of Petri dishes are prepared with equal quantities of the various media, at least two and preferably more dishes for each combination of species and concentration of antiseptic, and single colonies of the various species are planted centrally in the dishes. Rates of growth are recorded as colony diameters, usually with comments on the type of growth, such as density of mycelium and degree of sporangia. Fuller details of the method are given by Morris (1927). In this way a fair comparison between

different antiseptics may be achieved and some idea obtained of the amounts required to suppress growth. The method, although often the only one available for preliminary tests, is, however, open to the objection that such test media contain a very high percentage of water and the conditions are thus not comparable with those obtaining in practice. It is therefore necessary, before the true value of an antiseptic can be assessed, to make further tests under works conditions. In some industries, such as the manufacture of toilet preparations, incorporation of antiseptic with product can be carried out with very small batches of material and a number of tests can be run at comparatively little expense. Even here, however, the preliminary tests, as outlined above, are advisable, as they give results quickly, whereas experiments with industrial products for export may take months. With such materials as textiles, where the antiseptic is added to size or dressing, a single factory experiment may be costly and dislocating to normal work, so that it is absolutely necessary to obtain as much information as possible from laboratory tests.

It remains to give some details of the substances in common use as antiseptics in industry.

INORGANIC SUBSTANCES

Zinc chloride was originally suggested for the preservation of wood by Sir W. Burnett in 1838 (E.P. 7747). For this purpose it has been entirely supplanted by creosote preparations and copper compounds, but it has found extensive use in the cotton industry as an ingredient of the size added to the warp threads to assist weaving. It has to be used in fairly high concentrations, usually reckoned as 10 per cent on the weight of adhesive substances used in the size, but it is reasonably cheap and has the additional advantage of adding weight to the yarn. It is only moderately effective and, as stated above, may fail entirely when the goods are infected with certain *Aspergilli*, notably *A. terreus*.

Copper compounds. Copper sulphate has been suggested for use in various trades but has never been popular, possibly because of a much-repeated statement in trade literature that moulds have been found growing on strong solutions of the salt used for electrotyping. On the whole it is more efficient than

zinc chloride but is unreliable in the same way as the latter and is more expensive. Organic compounds of copper, such as copper naphthenate, have comparatively recently found extensive use for the preservation of timber. Copper carbonate in various forms, such as Burgundy and Bordeaux mixtures, are widely used for spraying plants as a preventive of mildew. Their use was originated by Pasteur at a time when the vineyards of France were threatened with ruin by the rapid spread of the vine mildew.

Mercury salts are little used, although quite effective, on account of their well-known poisonous nature and the fact that the metal is readily deposited from solutions of the salts by other common metals.

Thallium salts are powerful antiseptics but are little used, partly owing to high cost and partly due to the publicity given to one or two cases of accidental poisoning some years ago. Actually, although the price of thallium carbonate averages about 60 shillings per pound, the cost in practice compares very favourably with that of other antiseptics because of the low concentrations required.

Sodium silicofluoride behaves somewhat erratically but has enjoyed considerable popularity and constantly appears on the market under a variety of fancy names. One disadvantage for many purposes is its low solubility in water. It has been found to be one of the best antiseptics for the protection of raw rubber, the material being soaked in a 0.25 per cent solution.

Boric acid and Borax. Both substances are of low toxicity to most moulds but find a limited application in some industries where highly poisonous substances are debarred. They are used, either singly or in combination, as a wash for citrus fruits to minimize attack by the rot organisms, *Penicillium digitatum* and *P. italicum*. A number of papers relating to their use will be found in the various agricultural journals published by the exporting countries.

ORGANIC SUBSTANCES

The number of compounds having antiseptic value is very large. Many, however, are too expensive for most purposes,

whilst others have very limited applications owing to pronounced colour, odour, volatility or instability, with the result that there are only a comparatively small number of substances of real value to industry.

Creosote finds its main application for the preservation of wood. It gives considerable protection when merely brushed on, but, for adequate effect in timber which has to withstand severe conditions, it is applied under pressure, sometimes following vacuum, or at a temperature sufficiently high to boil the sap out of the wood. Its use for railway sleepers, pit props, piles and telegraph poles has meant increased safety for large numbers of workers as well as an enormous saving in the cost of replacements. Its somewhat objectionable odour, even when partially purified as in certain proprietary preparations, its dark colour and action on the skin, prevent its more general use.

Phenol is probably more efficient for preventing bacterial rather than mould growth. Nevertheless, it shows a fairly high toxicity to most fungi. In an acid medium its odour is distinctly noticeable and objectionable for some purposes, whilst as alkaline phenate its antiseptic value is lower. Some of the halogen-substituted phenols are much more efficient than the parent substance, but they are more volatile and have more pronounced odours.

Paranitrophenol is a valuable antiseptic for use in products in which its colour is no detriment. The free acid has a pale, though definite, yellow colour, whilst the alkaline salts are intensely yellow. It is used at a concentration of approximately 0.1 per cent, calculated on the weight of substance to be protected. It is largely used for treatment of rubber, the material being soaked for several hours in a 0.15 to 0.3 per cent solution.

Salicylic acid has been much recommended but has never proved entirely reliable in use. When tested in the laboratory against a representative collection of moulds, it has been found to inhibit some species completely, even in low concentration, whilst a few species, notably the black *Aspergilli*, are little or not at all affected, and, as stated above, can actually utilize the acid in a culture medium containing no other source of carbon.

Shirlan, the anilide of salicylic acid, has been patented as an antiseptic by the British Cotton Industry Research Association (1928). It was selected by them, after testing a very large number of organic compounds, as the most efficient antiseptic for use in the cotton industry, which demands an antiseptic which is colourless, odourless, non-volatile in steam and unaffected by other materials used in sizing and finishing or by contact with common metals. It is now finding increasing use in other industries because of its high efficiency and reasonable cost. It is marketed in two forms, the free acid (Shirlan), which is only slightly soluble in water, and the readily soluble sodium salt (Shirlan NA). It has been found that the minimum amount for adequate protection is approximately 0.04 per cent of the weight of substrate. This quantity is sufficient in what may be termed normal circumstances, but it is advisable to use double the amount if goods are likely to be exposed to a damp atmosphere.

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CHAPTER XIII

INDUSTRIAL USES OF FUNGI

As an offset to the incalculable damage caused by fungi there are a number of industrial processes in which the biochemical activities of moulds are turned to good account. Only a brief discussion of these will be entered into here and the reader is referred to the literature cited for fuller accounts. A few such processes have already been indicated in previous chapters but are mentioned again for convenience of reference.

Alcoholic Fermentation and Mould Enzymes. There are two industries, brewing and baking, which use processes of great antiquity, both dependent on the fact that yeasts convert sugar into alcohol and carbon dioxide. In the brewing of alcoholic beverages alcohol is the important product, whilst the carbon dioxide, once allowed to escape as useless, is now a valuable by-product, being collected, solidified and marketed as "dry ice." In baking, on the other hand the production of alcohol is incidental and it is the carbon dioxide which is valuable, causing the dough to rise and giving lightness to the bread. Both industries have an extensive literature of their own and need no further description here.

Yeasts secrete the enzyme zymase, which effects the conversion of sugar to alcohol, but lack diastase, the enzyme which breaks down starch to sugar. There are, however, a number of fungi which secrete a whole range of enzymes and can ferment complex carbohydrates without a preliminary saccharification. In certain processes for the production of alcoholic beverages from starchy materials moulds alone are used, but in others moulds effect the saccharification of the starch, after which a yeast is allowed to act on the sugars produced, since, although the mould can complete the conver-

sion to alcohol, the yield is better when yeast is used for the second stage. Fitz in 1873 was the first to show that alcohol is produced by a mould, *Mucor mucedo* (later identified as *M. racemosus*), and other workers have since shown that similar results are obtained with several other species of the same genus. In the "Amylo" process, which is still used in many countries in more or less modified forms, *Mucor Rouxii* was first used but was later replaced successively by various species of *Rhizopus*. Good general accounts of earlier practice are given by Lafar (1903) and Wehmer (1907), whilst more modern methods are described by Galle (1923). Various Mucoraceæ are also active agents in the so-called starters, used for initiating alcoholic fermentations and marketed in various Eastern countries, Chinese rice being the best known example.

Japanese "Koji" differs from other starters in making use of quite a different group of fungi, strains of the *Aspergillus flavus-oryzæ* series. In addition to the production of Saké, the national alcoholic beverage of Japan, a number of other industries utilize the enzymes secreted by *A. flavus-oryzæ*. Soy sauce is made by fermentation of soya beans, the starter being produced by growing the fungus on cooked beans, usually mixed with some other starchy material to aid rapid growth. As a result of an extensive study of the enzymes of this group of moulds Takamine has introduced into commerce a number of products of high enzymic activity, sold under a variety of names such as "Takadiastase" and "Polyzime," and used for the dextrinization of starch and the desizing of textiles. Takamine himself (1914) has summarized his work in a short paper, which should be consulted for further details.

Mould-ripened Cheese. The manufacture of mould-ripened cheese is another industry of unknown origin. Until recently such cheeses were associated with particular districts, such as the caves of Roquefort and the town of Stilton, and the methods used were entirely empirical, the distinct flavours of certain brands being dependent on a combination of slight local peculiarities in the quality of the curd and of a natural local infection of a particular strain of the all-important mould. It was not until 1906 that Thom's studies of the cheese moulds led to a proper understanding of the ripening process and, even

at the present day, when countless cheeses are being made by pure culture methods, there are certain local blue-veined cheeses whose production is a happy accident rather than a matter to be determined at choice, the nature and source of the marbling being a complete mystery to those who make the cheeses.

There are two distinct types of mould-ripened cheese, the soft cheeses of the Camembert and Brie types and the green or blue veined cheese, of which Roquefort, Gorgonzola and Stilton are the best known. In the first type the moulds concerned are *Penicillium camemberti* Thom and *P. caseicolum* Bainier, the two giving somewhat different flavours. The curd is made into cakes 3 to 4 cm. thick, salted on the surface and either inoculated with spores of the fungus or placed in an infected room. The initial moisture in the cakes is 55 to 60 per cent and the air in the ripening room is maintained at a temperature of 50–60° F. and a relative humidity of about 88 per cent. Freedom from infection by undesirable moulds depends on maintaining both temperature and humidity within fairly narrow limits. The mould grows on the surface of the cheese and gradually softens the whole mass of curd, the process requiring about four weeks for completion. The most serious source of infection and spoilage is *Scopulariopsis brevicaulis*, which gives to the cheese an ammoniacal taste and odour.

For production of the marbled cheese the raw curd is pressed so as to leave irregular cracks and channels. In the pure culture method a sterile curd is inoculated, before pressing, from a culture of the mould (usually on bread), and is later aerated from time to time during the ripening process by piercing with wires. In the older process natural infection is relied on, and the success of the method depends on the fact that few moulds other than *P. roqueforti* can grow with the small amount of oxygen contained in the narrow air spaces in the curd, and hence the chance of contamination with an undesirable species is small. Although some workers consider that distinct species are concerned in the ripening of the various types of cheese, Thom has shown that, for all practical purposes, all the strains may be regarded as one species. We still know little, however, of the effect of using different strains under otherwise identical conditions.

Oxalic and Citric Acids. There are a few modern processes which have been developed as the direct result of purely academic investigations into the biochemical activities of moulds. The first worker to make substantial progress in this field was C. Wehmer who, in 1891, showed that oxalic acid is a definite fermentation product of *Aspergillus niger* and made an extended study of its production from various sugars. Oxalic acid, however, has never been made commercially by this method because the more usual chemical methods are cheaper. In 1893 Wehmer described the production of citric acid by two species of moulds which were made the types of a new genus, *Citromyces* (now included in *Penicillium*). Other investigators have found that citric acid is a fermentation product of many species of *Penicillium*, but in no case is the yield sufficiently good to enable this method of production to compete with the established process of extracting the acid from citrus fruits. In 1917, however, Currie showed that, in the fermentation of sugar by black species of *Aspergillus* (the *A. niger* group) there is a distinct lag between the acidity of the medium due to oxalic acid and total acidity, the difference representing citric acid. Methods were worked out for suppressing the formation of oxalic acid and increasing the yield of citric acid, the chief essentials being a high initial concentration of sugar (about 15 per cent), low concentrations of ammonium nitrate as source of nitrogen, and an acid reaction of the culture medium, pH about 3.5. However, a number of difficulties have had to be overcome before such a process could be worked commercially. Sterilization of culture media is an expensive operation; an abundant supply of air is required and must be supplied in a sterile condition, or alternatively, the fermentation must be carried out in shallow layers of liquid with free aeration and suitable protection from infection, requiring expensive plant and expert supervision; and, perhaps most important of all, any organism is liable to be erratic in its behaviour, making the question of yields uncertain. A large number of patents have been taken out in this field, but the actual methods at present in use have not been made public in their entirety. Citric acid is certainly being made successfully in England, Belgium, the United States of America and Japan, in quantities sufficient to make these countries

almost or quite independent of imported acid made from citrus fruits.

Gluconic acid is formed from sugars by the action of a large number of species of moulds, chiefly species of *Aspergillus* and *Penicillium*, and a considerable amount of work has been carried out in the United States of America in an attempt to develop its large-scale production by mould fermentation (May *et al.*, 1927; Herrick and May, 1928; May *et al.*, 1929). Whether the process is yet being worked successfully on an industrial scale is, however, doubtful. The acid is used chiefly as the calcium salt, in place of calcium lactate, in medicinal and food preparations.

Gallic acid was obtained by Calmette in 1902 by fermenting a clear extract of tannin by means of an organism which he named *Aspergillus gallomyces*, the fungus being grown submerged in a well-aerated and agitated liquid. Modifications of Calmette's process have been used, and probably are still being used, both in Europe and in America, but exact data are not available.

Glycerol. About the middle of last century Pasteur (1859) showed that glycerol is formed in small amounts during the alcoholic fermentation of sugar by yeast, the maximum yield being about 3 per cent of the sugar consumed. During the World War the acute shortage of fats in Germany led to an investigation of the process with a view to increasing the production of glycerol. It was found that the yield may be much improved by carrying out the fermentation in an alkaline medium and still more so by adding to the culture medium sodium sulphite, which has the additional advantage that it inhibits the growth of bacteria without affecting the activity of the yeast. The yield of glycerol obtained was about 25 per cent of the sugar used and actually something over 1000 tons per month was produced by this method. After the War details of the process were published by Connstein and Lüdecke (1919). Later the technical production of fermentation glycerol was developed in the United States of America, using a special strain of yeast and adding sodium carbonate to the fermenting liquor up to a final concentration of approximately 5 per cent (Connstein and Lüdecke, 1924).

Fats are synthesized from carbohydrates by a large number of fungi. Until recently, however, only one species, a yeast usually known as *Endomyces vernalis* Ludwig, was of more than theoretical interest, it having been used for the large-scale production of fat in Germany towards the end of the War. According to Haehn and Kintto (1924), the essentials for successful working are a well-aerated medium rich in sugar and poor in nitrogen. They claim yields up to 25 per cent of the dry weight of the yeast cells and 30 per cent of the sugar consumed, also that the yield can be increased by the introduction of alcohol vapour in the air supply.

In the last few years a number of American workers (Lockwood *et al.*, 1934; Ward *et al.*, 1935) have investigated the production of fat from glucose by a large number of species of moulds. The largest amount is given by *Penicillium javanicum* van Beyma, which gives yields of fat up to more than 40 per cent of the weight of the mycelium, the best results being obtained with a medium containing 40 per cent of glucose. The process of production and isolation of the fat has been carried out with semi-large-scale apparatus and could no doubt be worked industrially in any emergency which created a shortage of fats from the usual sources.

Proteins are synthesized by a number of fungi, usually as cell constituents, more rarely in the substrate. In particular, baker's yeast and *Torula utilis* contain high percentages of protein which can be treated to give products resembling meat in appearance and flavour, and which are said to have high nutritive value. Since yeast can be grown on the large scale with ammonia as source of nitrogen and molasses as source of carbon, the economical manufacture of meat substitutes is not impossible, the idea of converting atmospheric nitrogen to protein being particularly attractive. At present, however, it is doubtful whether there is sufficient evidence to show that yeast is a complete substitute for animal protein in human diet, although it does form an excellent cattle-food and can thus be converted indirectly into first-class protein.

Vitamins. One of the best sources of the vitamin B complex is yeast, which is, in fact, one of the very few readily accessible foods containing all the four or more factors which comprise the vitamin B group. The increasing recognition of

the importance of these vitamins has led food manufacturers to put on the market a number of preparations of high potency, made from yeast extracts or autolysed yeast. The best known of these, used as a source of vitamin B in numerous nutritional experiments, is Marmite.

Ergosterol, the precursor of vitamin D, is synthesized by a number of moulds as well as by yeasts (Pruess *et al.*, 1931, 1932 *a* and *b*; Birkinshaw *et al.*, 1931) and there are on the market at the present time a number of preparations of irradiated ergosterol, mostly made from yeast.

Although ascorbic acid (vitamin C) has not yet been obtained as a metabolic product of fungi, substances closely related in chemical structure are produced by certain *Penicillia* (Clutterbuck *et al.*, 1935; Birkinshaw and Raistrick, 1936) and it is not outside the bounds of possibility that the vitamin itself will be found in due course.

Miscellaneous Products of Moulds. During recent years intensive investigations on the biochemistry of moulds have been carried out in several countries, with results which are of great theoretical interest and which may become the basis of important industrial processes in the future. In particular, H. Raistrick and co-workers (1931-7) have shown that a number of common moulds can produce from glucose a bewildering variety of complex organic compounds, many of which have not yet been synthesized by chemists. The majority of these are of purely academic interest at the present time, but the metabolic products of one genus of fungi appear to have a wider importance. Some species of *Helminthosporium* synthesize from glucose substituted anthraquinones of a type not readily made by purely chemical means (Charles *et al.*, 1933; Raistrick *et al.*, 1933, 1934), the yield in the case of *H. gramineum* Rabenhorst being as much as 30 per cent of the total weight of mycelium. The main factor which is likely to impede the large-scale production of these valuable dyestuffs intermediates is the extreme slowness of the fermentation, a difficulty which will probably be overcome by intensive cultural work.

A useful summary of our knowledge up to date of the biochemistry of moulds is given by Birkinshaw (1937). Other general literature relating to industrial fermentations is cited

in Chapter XIV. Ramsbottom (1936) deals with the uses of fungi in a much wider sense.

Fungi and the Soil. Amongst the uses of fungi, though hardly to be classed as an industrial use, should be mentioned the rôle they play in maintaining the fertility of the soil. Along with bacteria they are responsible for breaking down all kinds of dead organic matter to form humus, returning to the soil and the atmosphere the materials removed by other living things. Without such scavengers other forms of life would soon become extinct.

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CHAPTER XIV

MYCOLOGICAL LITERATURE

The following list of publications is not intended to be in any sense a complete bibliography. Apart from a number of works used for identification of fungi, the literature cited includes only such as is considered to be useful to a student whose interest extends to the more general aspects of mycology and who wishes to keep abreast of modern knowledge. Anyone who desires an exhaustive list of publications relating to any particular branch of the subject will find full bibliographies in the various monographs mentioned below.

Unfortunately a number of the most important mycological publications are to be found in only a few English libraries. Some of these may be obtained on loan through the various county libraries, but a few works of reference cannot be borrowed. These may, however, be consulted, by special permission, at the libraries of the British Museum (Natural History), the Imperial Mycological Institute, Kew, and the various universities (if available there).

1. GENERAL WORKS USED FOR IDENTIFICATION OF FUNGI

CLEMENTS, F. E., and SHEAR, C. L. (1931). *Genera of Fungi*. New York: The H. W. Wilson Co.

This is a second edition, much enlarged, of a book published by Clements in 1909 and intended primarily as a key to Saccardo's *Sylloge*. Keys are given to the known genera, with references to Saccardo, but there are no separate generic diagnoses, and, in consequence, the book is not so easy to use as Lindau's (see below). Although written in English a fair knowledge of Latin is essential to anyone using the book as the keys are liberally besprinkled with Anglicized Latin terms. There is, however, a fairly extensive glossary of Latin and English terms. The classification is, in

several respects, unorthodox and must be followed with caution by the beginner. A number of plates of illustrations are included, but these cover only a small selection of genera.

CORDA, A. C. I. (1837-54). *Icones fungorum hucusque cognitorum*. Prague.

A somewhat rare book containing beautiful illustrations of a large number of fungi, not always accurate in the light of present-day knowledge owing to the comparative crudity of microscopes in Corda's time, but often helpful.

ENGLER, A., and PRANTL, K. (1897-1900). *Die natürlichen Pflanzenfamilien*. I Teil, Abteilungen 1 and 1*. Leipzig.

The work is accessible in most botanical libraries and is much used by taxonomists. It gives descriptions and illustrations of most genera but no separate descriptions of species.

LINDAU, G. (1922). *Kryptogamenflora für Anfänger*. Band II. Die mikroskopischen Pilze. 2nd ed., Berlin: Julius Springer.

Published in two parts, Part 1 including Myxomycetes, Phycomycetes and Ascomycetes, Part 2 including Rusts, Smuts and Fungi Imperfecti. This is a most useful and comparatively inexpensive book, apparently very little known in this country. It gives keys to all the families and genera, with separate generic diagnoses and descriptions of several species in most cases. The lists of species are, necessarily, in a book of this size, incomplete and do not always include the most common forms, but for tracking down a fungus to its genus the book is extremely good. The illustrations are not so good. They are all from line drawings, small, often very sketchy and not infrequently inadequate. No legends are given and magnifications are not stated, so that often a fair knowledge of structure is required in order to know what they are supposed to represent. They are, nevertheless, at times very useful.

RABENHORST, L. (1884-1921). *Kryptogamenflora von Deutschland, Oesterreich und der Schweiz*. Leipzig: Eduard Kummer.

A series of many volumes, illustrated, including not only the Fungi but other Cryptogams. Some portions are not yet completed. Band I, *Die Pilze*, is in ten volumes, of which the most useful to a student of moulds are Vol. 4, *Phycomycetes*, by A. Fischer, 1892, and Vols. 8 and 9, *Hyphomycetes*, by G. Lindau, 1904-10.

"Rabenhorst" is to be found in many libraries which do not contain a set of Saccardo's *Sylloge* and is a most useful work of reference. The lists of species are fairly complete up to the dates of publication and include most of those commonly occurring.

SACCARDO, P. A. (1882-1931). *Sylloge fungorum omnium hucusque cognitorum*. 25 volumes, to be continued. Pavia, Italy.

This unique work includes descriptions, in Latin, of all known species of fungi. It was originally planned in eight volumes, which were completed in 1889. By this time, however, the literature of descriptive mycology was growing to such an extent that a series of supplementary volumes was issued at intervals of a few years.

Since Saccardo's death in 1920 the publication has been continued by A. Trotter.

"Saccardo" is to the mycologist very much what "Beilstein" is to the organic chemist, often indispensable but always, of necessity, several years out of date. Unfortunately the work is very expensive and complete sets of volumes are to be found in only a few libraries in this country.

From the point of view of handy reference it is unfortunate, but unavoidable, that the species of a single genus are scattered through several volumes. The work is, however, well indexed and, with the requisite knowledge of Latin, not difficult to use. The main weakness is that many of the descriptions are, through no fault of Saccardo's, incomplete or merely fragmentary. Even at the time when Saccardo began the compilation it was impossible for one man to have first-hand knowledge of all the thousands of species of fungi. To a large extent, therefore, the treatment is non-critical, many of the diagnoses being merely transcriptions into Latin of original descriptions which were totally inadequate.

SACCARDO, P. A. (1877-86). *Fungi Italici autographice delineati*. Pavia, Italy.

A collection of illustrations, mostly coloured, of a very large number of fungi, including most of the common moulds.

2. MONOGRAPHS AND IMPORTANT PAPERS DEALING WITH PARTICULAR GENERA OF MOULDS

Alternaria

ELLIOTT, J. A. (1917). Taxonomic characters of the genera *Alternaria* and *Macrosporium*. *Amer. Jour. of Bot.*, 4, 439-76.

The best account so far of the genus *Alternaria*. Species of this genus are not difficult to identify if the author's conceptions of species limitations are adopted.

Aspergillus

THOM, C., and CHURCH, M. B. (1926). *The Aspergilli*. Baltimore: The Williams & Wilkins Co.

As indicated in Chapter VII this is the standard work on the genus. The least satisfactory sections are the chapters on the *A. glaucus* and *A. penicilloides* groups, but these will probably be brought into line with the rest in a second edition.

Fusarium

WOLLENWEBER, H. W., and REINKING, O. A. (1935). *Die Fusarien*. Berlin: Paul Parey.

Wollenweber's monograph of the genus, the first part of which was published in 1931 (*Zeit. f. Parasitenkunde*), has not been finished. In the meantime, the present book gives a somewhat simplified account of the genus and, although it is too soon to judge adequately, it offers hope of mycologists being able to identify *Fusaria* isolated from sources other than diseased plants.

WOLLENWEBER, H. W., SHERBAKOFF, C. D., REINKING, O. A., JOHANN, H., and BAILEY, A. A. (1925). Fundamentals for taxonomic studies of *Fusarium*. *Jour. Agric. Res.*, **30**, 833-43.

This paper still remains of importance to anyone working with *Fusaria*. It gives explanations of terms and criteria, and details of culture media, a good deal of the information not being included in *Die Fusarien*.

Mucorales

ZYCHA, H. (1935). Kryptogamenflora der Mark Brandenburg. Band VIa. Pilze II, Mucorineæ. Leipzig: Gebrüder Borntraeger.

This monograph is mainly taxonomic and includes descriptions of the known species of all the genera of Mucorales. The treatment of the two commonest genera, *Mucor* and *Rhizopus*, is excellent. The book is likely to supersede all previous publications used for identification of moulds belonging to this order.

Penicillium

THOM, C. (1930). The *Penicillia*. London: Baillière, Tindall & Cox. This is undoubtedly the most complete and satisfactory account of this genus up to date. It is not an easy book to use but is indispensable to anyone whose work entails regular identifications of *Penicillia*. A fair number of new species of *Penicillium* have been described since 1930, but the majority of these can be traced through the catalogues issued by the various national collections of cultures.

Yeasts

LODDER, J. (1934). Die anaskosporogenen Hefen, Erste Hälfte. Verhandelingen der Koninklijke Akademie van Wetenschappen te Amsterdam, Afd. Natuurkunde, (Tweede Sectie) Deel **32**, 1-256.

STELLING-DEKKER, N. M. (1931). Die sporogenen Hefen. *Ibid.*, Deel **28**, 1-547.

These two publications form parts of a comprehensive treatise on the Yeasts and yeast-like fungi, by workers at the Centraalbureau voor Schimmelcultures, Baarn, Holland. To those who wish to identify organisms belonging to these very difficult groups they are indispensable.

3. BOOKS ON GENERAL AND SYSTEMATIC MYCOLOGY

DE BARY, A. (1887). Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria. English translation. Oxford Univ. Press.

Although written fifty years ago, this is still one of the best accounts of systematic mycology.

BESSEY, E. A. (1935). A Text-book of Mycology. Philadelphia: P. Blakiston, Son & Co.

This covers much the same ground as other modern books on the

subject but contains in addition an excellent bibliography under the title "Guide to the literature for the identification of fungi."

FITZPATRICK, H. M. (1930). *The Lower Fungi—Phycomycetes*. New York: The McGraw-Hill Book Co.

A very full account of the comparative morphology of this class of fungi.

GÄUMANN, E. A., and DODGE, C. W. (1928). *Comparative Morphology of Fungi*. New York: The McGraw-Hill Book Co.

Although regarded as a standard work on the subject, this is not a book for the beginner and should not be attempted by the student until he has acquired a fairly good working knowledge of mycology.

GUILLIERMOND, A., and TANNER, F. W. (1920). *The Yeasts*. New York: John Wiley & Sons, Inc.

An English translation by Tanner of *Les Levures*, published by Guilliermond in 1912, considerably enlarged and brought up to date in collaboration with the author. It has long been regarded as the standard systematic treatment of the sporing yeasts. (See Stelling-Dekker.)

GWYNNE-VAUGHAN, H. C. I., and BARNES, B. (1937). *The Fungi*. 2nd ed. Cambridge Univ. Press.

For the beginner this is a much more readable book than Gäumann and Dodge. The classification adopted differs somewhat from that of Gäumann and also from that of Bessey.

RAMSBOTTOM, J. (1929). *Fungi*. London: Ernest Benn (sixpenny series).

A popular account of the Fungi from many aspects. It is a most useful introduction to the subject and should be read by everyone commencing the study of mycology.

STELLING-DEKKER, N. M. (1931). *Loc. cit.*

This is the most recent treatment of the sporing yeasts. The classification differs somewhat from that of Guilliermond and Tanner.

4. BOOKS ON APPLIED MYCOLOGY

HARSHBERGER, J. W. (1917). *Mycology and Plant Pathology*. Philadelphia: P. Blakiston, Son & Co.

Primarily a book on plant pathology, this has been much used as an introduction to the study of moulds in default of a work devoted specifically to such. It gives a brief account of systematic mycology and also, in a series of appendices, a number of keys (not always quite accurate) to *Mucor*, *Aspergillus* and *Penicillium*.

HENRICI, A. T. (1930). *Molds, Yeasts and Actinomycetes*. New York: John Wiley & Sons; London: Chapman & Hall.

The consideration of moulds and yeasts from an industrial standpoint is subordinated to the medical aspect of mycology. As an introduction to the study of pathogenic fungi and of that diffi-

cult class of organisms, the Actinomycetes, the book can be recommended.

LAFAR, F. (1904-14). *Handbuch der technischen Mykologie*. Zweite Aufl. Jena: Gustav Fischer.

A series of five volumes dealing with the rôle of micro-organisms in industry. Vol. 1 (1904-7) deals with general morphology and physiology of organisms used in fermentations; Vol. 2 (1905-8) with the food industries; Vol. 3 (1904-6) with water, soil and manure; Vol. 4 (1905-7) with special morphology and physiology of yeasts, and mould fungi; Vol. 5 (1905-14) with the mycology of beer, spirits, wines, fruits, vinegar, leather and tobacco. Recognized as a standard work on the subject.

LAFAR, F. (1898-1910). *Technical Mycology*. English translation of first German edition, by C. T. C. Salter. London: C. Griffin & Co.

Vol. 1 (1898) deals with bacterial fermentations; Vol. 2, Part 1 (1903), Part 2 (1910) with fermentations brought about by fungi. Whilst not so full or up to date as the later German edition, this gives a good account of the history of the fermentation industries and of the technique of last century.

SMITH, H. F., and OBOLD, W. L. (1930). *Industrial Microbiology*. London: Baillière, Tindall & Cox.

Gives accounts, many of them very brief, of industrial processes utilizing bacteria, yeasts and moulds, including some very modern methods.

THAYSEN, A. C., and BUNKER, H. J. (1927). *The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums*. Oxford University Press.

THAYSEN, A. C., and GALLOWAY, L. D. (1930). *The Microbiology of Starch and Sugars*. Oxford University Press.

These two books give descriptions of a large number of organisms, including bacteria, Actinomycetes and moulds, and summarize the results of researches into the rôle of these organisms in the decomposition of the various complex carbohydrates.

VARIOUS AUTHORS (1930). *Industrial Fermentations*. *Ind. and Eng. Chem.*, **22**, 1148-1225.

This is a series of papers presented at a symposium held at Cincinnati in September, 1930, and dealing with both bacterial and mould fermentations. It is a very valuable résumé of modern practice. (Obtainable as a separate reprint.)

5. JOURNALS AND PERIODICALS

The following list does not include all the journals devoted to mycology but only those which regularly contain papers of interest to the student of moulds and which are reasonably accessible.

Annales Mycologici. Berlin.

Contains original papers, mostly on taxonomy of all classes of fungi, and lists of new literature but not abstracts.

Biological Abstracts. Baltimore, Md., U.S.A.

"Published under the auspices of the Union of American Biological Societies, with the co-operation of biologists generally." This is a continuation of *Botanical Abstracts*, published 1918-26. It is issued monthly, October to May, and bi-monthly June to September, and covers all branches of biological sciences. Abstracts are classified and many of them are written by the authors of the papers abstracted.

Bulletin Trimestriel de la Société Mycologique de France. Paris.

A quarterly journal with a preponderance of papers on taxonomy. Includes abstracts.

Hedwigia. Dresden.

Issued monthly. Contains original papers, relating to Cryptogamic Botany in general and to plant pathology, and excellent abstracts. A half-yearly supplement contains lists of titles of new literature and book reviews.

Journal of Agricultural Research. Washington, D.C. Government Printing Office.

Published twice monthly; two volumes per year. Although it is concerned primarily with all branches of agriculture, a considerable number of papers on taxonomy of moulds have appeared in this journal.

Mycologia. Lancaster, Pa.

This was originally, 1885-1908, *The Journal of Mycology*. It is published bi-monthly for the New York Botanical Garden and has been since 1933 the official organ of the Mycological Society of America. It covers all branches of mycology with a preponderance of papers on taxonomy. No abstracts.

Mykologische Zentralblatt. Jena.

A journal of abstracts, unfortunately issued only from 1912 to 1915.

Review of Applied Mycology.

Issued by the Imperial Mycological Institute, Kew, and published monthly. A journal of abstracts only, covering all branches of applied mycology but not pure mycology.

Transactions of the British Mycological Society. London.

Four parts per year are issued, often two parts together. Contains original papers on all aspects of mycology. No abstracts.

Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Zweite Abt. Jena.

Issued monthly, with two volumes per year. Contains original papers in German and English, including a fair number on taxonomy of moulds, and classified abstracts in German. (*Erste Abt., Originale* is concerned with medical and veterinary bacteriology.)

Apart from the journals devoted to mycology, papers on fungi are frequently published in the various botanical journals and in journals devoted to particular industries, whilst articles on applied mycology are to be found in numerous trade periodicals. All of these, however, are adequately covered by the various journals which publish abstracts.

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